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Editor
ROY WALDO MINER

PARENTAL AGE AND CHARACTERISTICS OF THE OFFSPRING

BY

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PARENTAL AGE AND CHARACTERISTICS OF THE OFFSPRING*

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* This series of papers is the result of a Conference on *Parental Age and Characteristics of the Offspring* held by the Section of Biology of The New York Academy of Sciences, January 23 and 24, 1953.

INTRODUCTION TO PARENTAL AGE AND CHARACTERISTICS OF THE OFFSPRING

By E. V. Cowdry
Washington University, St. Louis, Mo.

The New York Academy of Sciences has to its credit the habit of picking important problems for its conferences and monographs, as well as organizing discussions to such good effect that they are of pioneering value. To analyze the influence of parental age on the offspring is obviously of interest to all of us. Pearson, Pearl, and others observed that a hereditary influence sometimes conditions longevity, though accidents often intervene. But we need to discover much more.

The first question is: how much do the ages of the maternal and paternal parents contribute to the length of life of the male and female offspring? Another is: what are the ways in which the influences operate? We think at once of inheritance through nuclear and cytoplasmic components, and of maternal influence *in utero*. At the human level, the impact on the developing personalities of the offspring exercised by elderly, instead of by young, parents, may perhaps force different kinds of adjustments of consequence in later life. In relatively long-lived men and women "each organ system has a characteristic time curve for its breakdown, differing from the curve of any other system" (Pearl).

This being the case, we should scan the cardiovascular system for evidence of inherited strength or weakness. This system deserves special attention because over half of the deaths in the United States are attributable to its failure in one way or another. These deaths far surpass those from cancer and may follow long periods of invalidism with inevitable social and economic distress.

In this monograph it is necessary to evaluate evidence springing from many kinds of advances, for we are dealing with the operation of fundamental vital processes which can be investigated chemically, physiologically, genetically, and in numerous other ways. All living things have hereditary endowments, must adjust themselves to environments, must age and die. Experimentation is far easier with some than with others. Particularly desirable in the choice of material for investigation are simplicity of organization, feasibility of control of environment, and short life-span permitting the study through an adequate number of generations of sufficiently large numbers of individuals to yield statistically valid observations.

One naturally expects, in a monograph such as this, to witness the cross fertilization of ideas. A clue found in plants, for instance, may well reveal an opportunity for significant work in protozoa, in mice and in men, or vice versa. Cooperation here achieved will gain strength and spread. A few of the observations and experiments involving teamwork will bring forth fruit promptly. Others will do so years later. Ours is a long-term kind of research. We promise nothing, but our hopes are high.

The papers and discussions embodied in this monograph will make biological history. The conference on which this mongraph is based is the first that has focussed so directly and so accurately on parental age and the characteristics of the offspring. When the conference was first proposed by Doctor Strong, I think that the general reaction was one of doubt that sufficient lines of advance had developed enough material awaiting integration to warrant this international getting together. But the well-constructed program before us is evidence of the superiority of his vision not only of the importance of the problem, but also of the fact that it is now ripe for consideration.

A NONGENIC FACTOR IN THE LONGEVITY OF ROTIFERS

By Albert I. Lansing
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It is a curious thing, that, although much progress has been made on many fronts in genetics, enzymology, infectious diseases, cancer biology, *etc.*, remarkably little is known of the processes of aging. Except for the generalization, not too well documented, that genetic constitution and environmental stresses operate to determine longevity, there is little of a concrete nature available for the characterization of the aging process. A precise definition cannot be formulated, nor can a working description of structural, chemical, or physiological age changes be outlined. Indeed, it is a matter of speculation as to when aging really begins.

We are essentially in the same position as a physician confronted with a patient suffering from an unknown disease, with undefined symptoms, and with no information as to the time course of the disease. In a situation such as this, there is little to be done but to observe. And so with aging, we may, through accumulation of biological data, eventually arrive at a rational pattern of symptoms to diagnose senescence.

At the moment we are concerned with the influence of maternal age on characteristics of offspring. Such influences are more than biological curiosities. If they exist, as apparently they do, we come headlong on the fact that the maternal germplasm of even a homozygous individual changes with time. Here, then, is a source of inheritance of individual diversities with profound ecological and evolutionary implications. Further, if there is a critical maternal age at which effects are transmitted to the offspring it may be possible to use this as a marker for the time at which an individual begins to age. Still further, if it can be demonstrated that effects of maternal age can be transmitted to offspring through the ovum, in the absence of genetic and environmental variations we have clear evidence that nongenic factors operate in aging. So may open new vistas for study.

To my knowledge, the first indication that maternal age influences longevity and other characteristics of offspring through a nongenic mechanism is contained in the work of Jennings and Lynch in 1928. Using laboratory cultured clones of rotifers (parthenogenetic and hence, homozygous) Jennings concluded: "In sum, the individuals of a clone of *Proales sordida* are not all intrinsically alike. They are diverse in fecundity and in the length of certain of the life periods: in dependence on the size of the eggs from which they come, and through the latter, in dependence on the age of their parents. To obtain an intrinsically uniform population, eggs must be taken from parents of the same age and before old age has produced variability of the eggs."

Two years later, Sonneborn (1930), one of Jennings' students, published his studies on the inheritance of individual diversities in *Stenostomum incaudatum*, a flat worm. Although this investigation was not planned to deal with problems of aging, it joins with McCay's brilliant starvation study on rats

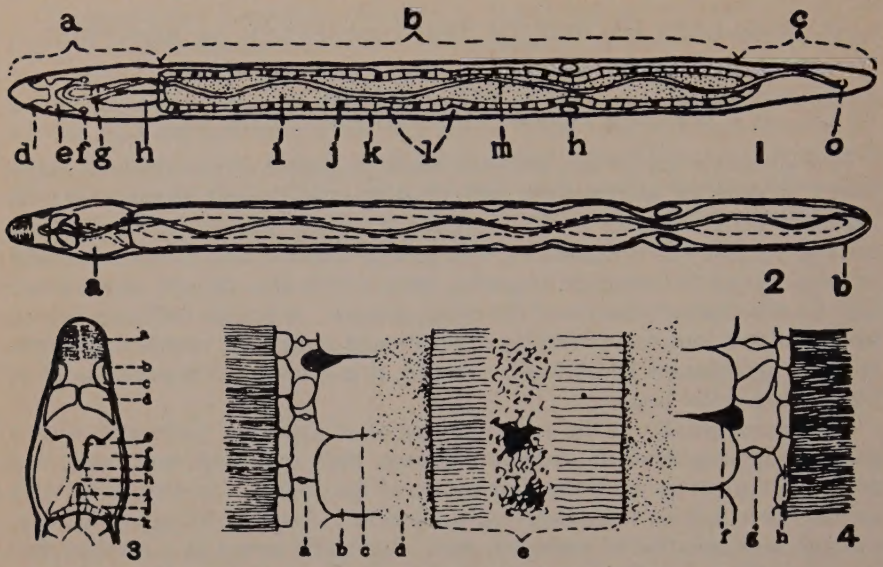

FIGURE 1. (1) Diagram showing characters commonly found in the genus *Stenostomum*. *a*, head; *b*, trunk *c*, tail; *d*, ciliated pit; *e*, cerebral ganglion; *f*, light-refractive organ; *g*, mouth; *h*, pharynx; *i*, intestine; *j*, intestina gland; *k*, pseudocoel; *l*, constrictions of intestine marking places where new heads are just beginning to form; *m*, protonephridium; *n*, anlage of cerebral ganglion of new zooid; *o*, nephridiopore. (2) Diagram of *S. incaudatum* showing specific differentiae (magnification, 70 diameters). *a*, pharynx, short, wide, and lacking glands. *b*, posterior part of trunk, lacking tail. (3) Head of *S. incaudatum* (magnification, 175 diameters). *a*, transverse muscles; *b*, ciliated pit; *c*, ciliated-pit ganglion; *d*, cerebral ganglion; *e*, mouth; *f*, pharynx; *g*, neck of flask-shaped lumen of pharynx; *h*, lumen of pharynx; *i*, sphincter in pyramidal mass extending from posterior wall of pharynx; *j*, base of flask-shaped lumen of pharynx; *k*, posterior wall of pharynx. (4) Diagram of longitudinal section through part of trunk (magnification, 1000 diameters). *a*, pseudocoel; *b*, globule; *c*, distal part of intestinal cell; *d*, proximal part of intestinal cell full of food in process of digestion; *e*, ciliated lumen of intestine, containing food and faeces; *f*, intestinal gland; *g*, transverse muscle; *h*, ciliated epidermis. (From T. M. Sonneborn. 1930. Structural characteristics of *S. incaudatum*. J. Exp. Zoöl. **57**: 57–108.)

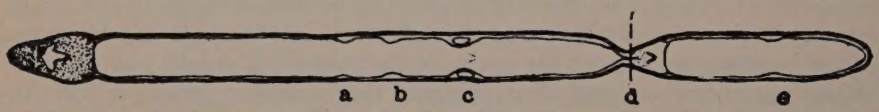

FIGURE 2. Fission characteristics of *S. incaudatum*. Chain of zooids about to divide at plane indicated by broken line (*d*). Magnification, 70 diameters. *a*, *b*, *c*, *d*, *e*, constriction of the intestine marking regions in which heads of zooids are developing. (From Sonneborn, *loc. cit.*)

(1934, 1939) to make the two keystone investigations in gerontology to date.

In its asexual reproduction, Stenostomum multiplies by transverse fission to form an anterior and posterior product of fission (FIGURE 2). An essential difference between the two daughter worms results from the anatomy of Stenostomum (FIGURE 1).

As Sonneborn noted, "When *S. incaudatum* divides . . . , the head and anterior part of the trunk pass into the anterior product of division, and the posterior part of the trunk passes into the posterior product of division. This same distribution of material occurs in all divisions. The head and trunk differ in that the growth of the former is definitely limited, whereas the latter continues to grow as long as asexual reproduction continues. The same head

goes into the anterior product of successive divisions. The trunk, on the other hand, continuously growing by proliferation of its tissues, is not the same during successive divisions. Throughout vegetative reproduction, successive anteriors retain repeatedly the most anterior fractions of this increasing mass; successive posteriors retain repeatedly the most posterior fractions of the increasing mass. So successive anteriors are alike in that all receive the same head; they are different in that each possesses a newly formed posterior part of the trunk. In successive posteriors, the head and anterior part of the trunk are newly formed in each individual, but the posterior part of the trunk is the same in all individuals."

The essential difference, then, between anterior and posterior fission products is in their growth activities; anterior products undergo little growth while posterior products, in regenerating most of the body organs, grow actively. Sonneborn, by repeated selection, established contrasting lines of anterior and posterior products through a series of generations (FIGURE 3). The differences in longevity of these lines is strikingly apparent (TABLE 1). The mean life span of all the anterior lines was 35 days while four posterior lines that died had a mean life span of 64.5 days, two lines that were lost accidentally were observed for 77 days, and two lines were observed for 115 days at which time they were discarded. This study suggests the working hypothesis that actively growing tissues do not age and that, conversely, nongrowing tissues age.

A number of years later, as a student of Sonneborn, it occurred to me that one might adapt the design of Sonneborn's investigation to a sexually reproducing animal, such as the rotifer. In essence, I set out to determine if there is a difference in longevity between progeny derived from eggs laid by actively growing and full-grown or old mothers.

The rotifer is particularly well adapted to such an analysis for a number of reasons. Rotifers reproduce parthenogenetically and so it is a simple matter to develop a genetically homozygous clone from a single female. The span of life of most species of this microscopic fresh water organism varies between one week and one month, making it readily feasible to observe whole life histories without committing a human life span to the project. Culturing methods are available to insure standardized environmental conditions. Buffered artificial pond water may be maintained at constant temperature and the rotifers may be fed a diet of *Chlorella vulgaris* cultured on agar slants. Although rotifers are less than one millimeter in length, their bodies contain a variety of organs including ciliated epithelium, muscle fibers, an ovary, intestinal tract, a primitive brain, and other differentiated organs (FIGURE 4).

Maternal age and longevity. The design of the experiments is illustrated in FIGURE 5. To initiate the investigation, a large number of eggs laid on a single day were isolated from a group of wild stock but homozygous mothers. These eggs were allowed to hatch and placed in isolation culture to establish the parental stock of the various series. In the species illustrated, the rotifers are adolescent on the second day of life, reach maturity on the third day, and begin to decline on the fifth day. Thus, to establish a line with actively growing mothers, one isolates the eggs laid on the second day of life. When this F_1 reaches its second day of life, the eggs laid on that day are isolated to establish an F_2

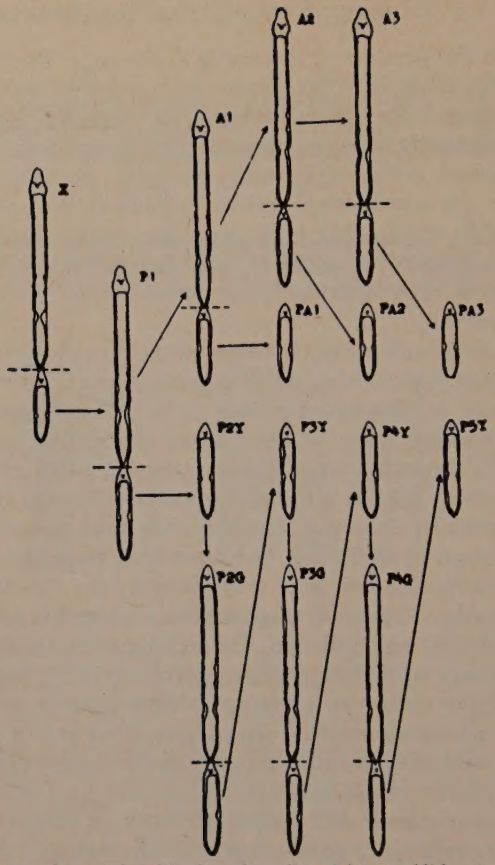

FIGURE 3. Selection procedure employed by Sonneborn (*loc. cit.*) to establish anterior and posterior lines. Method of selecting lines of anteriors and lines of posteriors, showing difference in size between posteriors produced by anteriors and posteriors produced by posteriors. X. Ancestor of both lines. P1, P2Y, P2G, P3Y, P3G, P4Y, P4G, P5Y. A line of posteriors. P2Y, P3Y, P4Y, P5Y. Young posterior individuals just produced by division of individuals in the posterior line. P2G, P3G, P4G. Grown posteriors about to divide. P1, A1, A2, A3. A line of anteriors. PA1, PA2, PA3. Young posterior individuals just produced by division of individuals in the anterior line. Broken lines indicate planes of division.

TABLE 1.

COMPARISON OF MEAN LENGTH OF LIFE OF LINES OF ANTERIORS WITH MEAN LENGTH OF LIFE OF LINES OF POSTERIORS

Lines of anteriors			Lines of posteriors		
	Number of lines	Mean length of life (in days)		Number of lines	Mean length of life (in days)
All	388	35.0 ± 1.1	All	8	79.8+
Group 5A (group with highest mean)	47	68.9 ± 2.0	Those which died	4	63.5
			Those which were lost	2	77.0
			Those which were discarded	2	115.0+

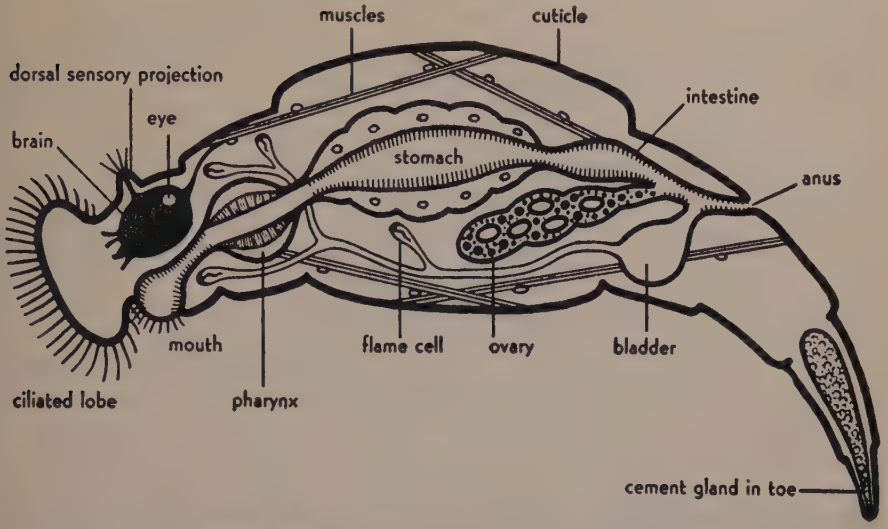

FIGURE 4. Outline schematic structure of a rotifer, showing structure. Only a few of the nuclei are shown (*e.g.*, muscles and stomach wall). (From Buchsbaum. 1938. Animals Without Backbones. Univ. of Chicago Press.)

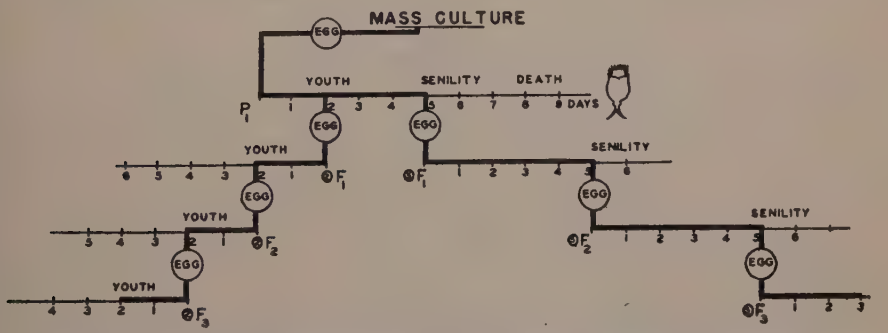

FIGURE 5. Schematic outline of procedure used to establish several generations of rotifers with constant parental age in each generation.

from adolescent mothers. This procedure may be repeated indefinitely. Each generation, of course, is placed in isolation culture and observed daily until death of the animals. Successive, or serial, generations of animals with uniform parental age in each generation form an orthoclone.

On the other hand, to observe the effects of having old ancestors, one isolates eggs laid on the fifth or sixth day of life of the parental stock. This F_1 is isolated as before and eggs are collected on the fifth or sixth day to initiate an F_2, then an F_3, and so on. All the animals are observed through their full life spans and records are maintained on fecundity and time of death.

FIGURE 6 graphically presents the data on mean life spans of several generations of lines from adolescent, middle-aged, and old mothers of the species, *Philodina citrina*. Adolescence in this species is reached on the fifth day, adult-

hood on the sixth day, and obvious senility on the fourteenth to fifteenth days. As the data show, mean life spans in the adolescent line slowly but significantly increase over seven generations while middle-aged and senile lines decline and die out over five and three generations respectively.

This pattern of result was consistent through numerous different experiments using three species of rotifers. Mean life span of offspring of adult or senile mothers is reduced. Second generation offspring of older mothers have still shorter life spans, and so on until extinction of the line. The number of generations required to reach such extinction depends upon the age of the ancestry involved.

In the absence of genetic variation and environmental fluctuations and with maintenance of both internal and parallel control lines, there seems no doubt but that something is transmitted through the eggs of adult or old mothers which accelerates aging in the offspring. This effect is not only transmissible but cumulative.

Since longevity of offspring of growing mothers is increased rather than decreased, it follows that either the aging factor is not operative or is lacking in eggs of adolescent females.

The obvious experiment at this point was to attempt to reverse the accelerated aging of late born of late born by switching for the third generation to early born selection. Such an experiment is graphically illustrated in

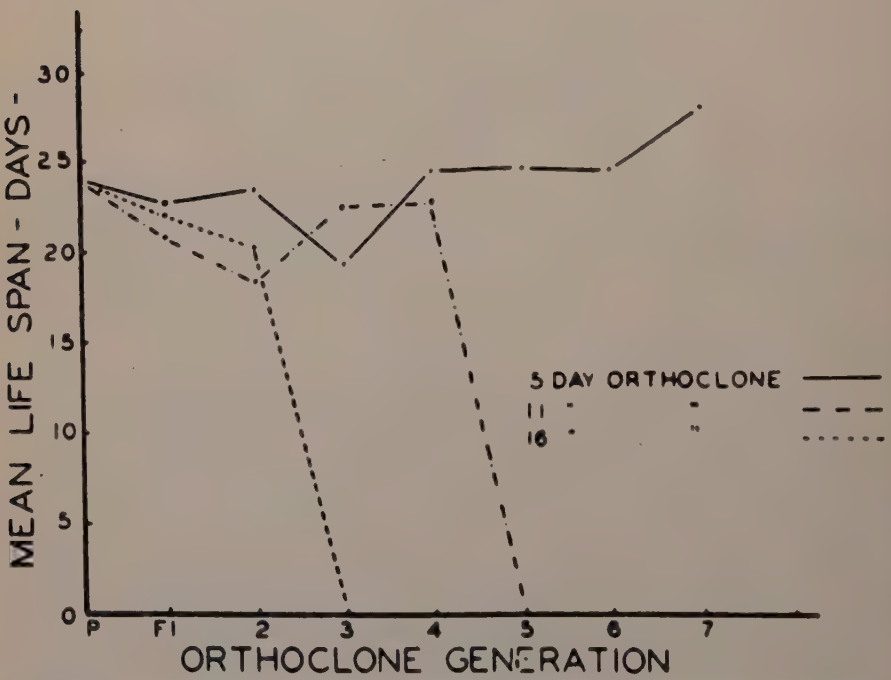

FIGURE 6. Graphic representation of life span differences between lines of rotifers derived from young and old mothers.

FIGURE 7. After two generations of senile parentage, an offshoot line was established from adolescent animals while a third generation of senile parentage was also isolated. The latter produced nonviable eggs while the former survived and through continued selection of eggs from growing mothers showed progressive increase in longevity through three generations. During the second generation an offshoot line of eggs from eight-day-old (adult) mothers was started and maintained for eight generations at which time the line died out. Still another offshoot line, now of eggs from adolescent mothers, was started in the fourth generation of the declining series and again the aging trend was reversed.

From this and several similar experiments, it was concluded that the acceleration of aging apparent in late born animals may be reversed. This observation eliminates the possibility that we are dealing with a genic mutation. Apparently a nongenic factor operates here to accelerate aging.

Maternal age and growth characteristics. That there is a close association between the growth status of the individual and its aging is further supported by the following studies. The previously described experiments showed that growing rotifers do not contain the aging factor while middle-aged and old rotifers do contain it. It also appeared that the greater the maternal age, the more marked was the suppression of longevity in the offspring and hence the fewer the number of generations that were required to produce extinction of the line.

Thus, orthoclones of 16- and 17-day-old mothers die out in three generations, of 11-day-old mothers die out in three generations, of 11-day-old mothers die out in four generations, and of eight-day-old mothers die out in eight generations. At the same time, it is to be recalled that orthoclones derived from

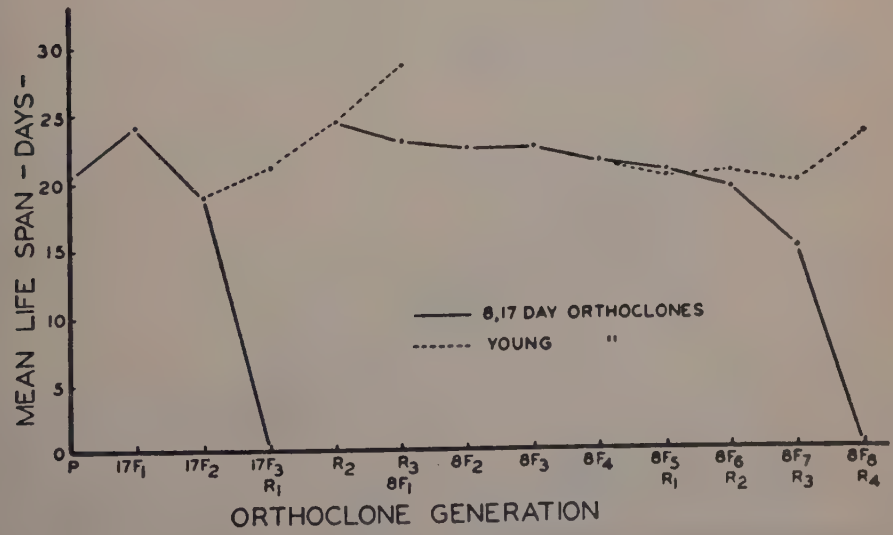

FIGURE 7. Longevity differences between young and old lines of rotifers and the reversibility of accelerated aging.

adolescent (five-day-old) mothers exhibit ever increasing longevity. One series was maintained through 54 generations with a gradual increase in longevity from 24 days to as much as 104 days. It is to be presumed that adolescent orthoclones survive an infinite number of generations. Appearance, therefore, of the aging factor and its limiting effect on longevity of both individuals and lines must make its appearance on either the sixth or seventh day of life. If it appears on the sixth day (when the rotifers attain adulthood), there would seem to be a direct association between growth cessation and aging.

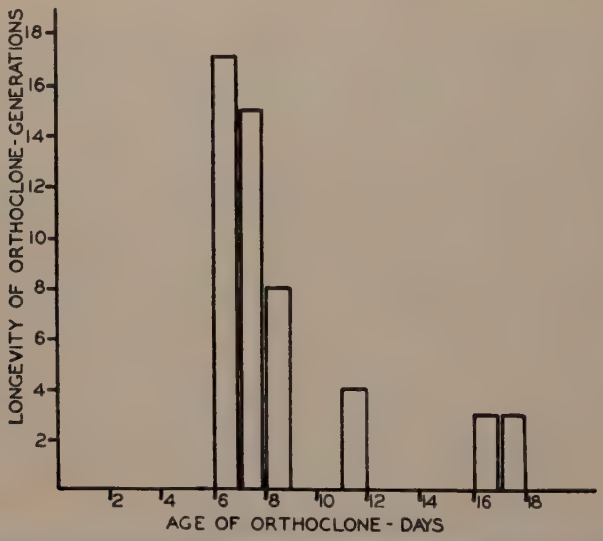

FIGURE 8. Histogram summarizing data on longevity of orthoclones with respect to maternal age. The five day orthoclone though not represented would presumably survive an infinite number of generations.

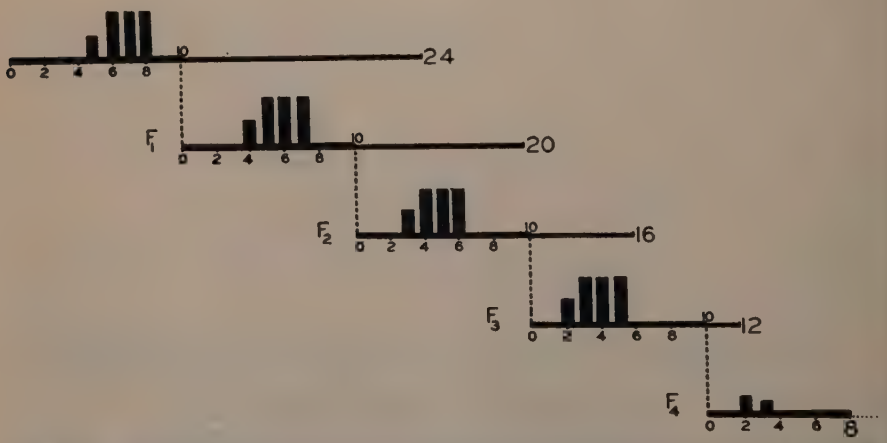

FIGURE 9. Histogram schematically showing that both time of egg production and rate of egg production are altered in old orthoclones.

Orthoclones of *Philodina citrina* at six and seven days were established and observed with the usual internal and parallel controls. The seven-day orthoclone manifested a gradual decline in longevity through a number of generations and died out in the fifteenth generation. Lastly, and most significant, the six-day orthoclone slowly but steadily declined and became extinct in the seventeenth generation (FIGURE 8).

Further evidence that growth and aging are closely linked is contained in the data on rate and time of onset of egg production. Such data for an old orthoclone are partially schematically shown in FIGURE 9. Mean life span drops steadily in each descendent generation. As longevity decreases, there is a shift in time of onset of egg production to earlier ages and maximal rate of egg

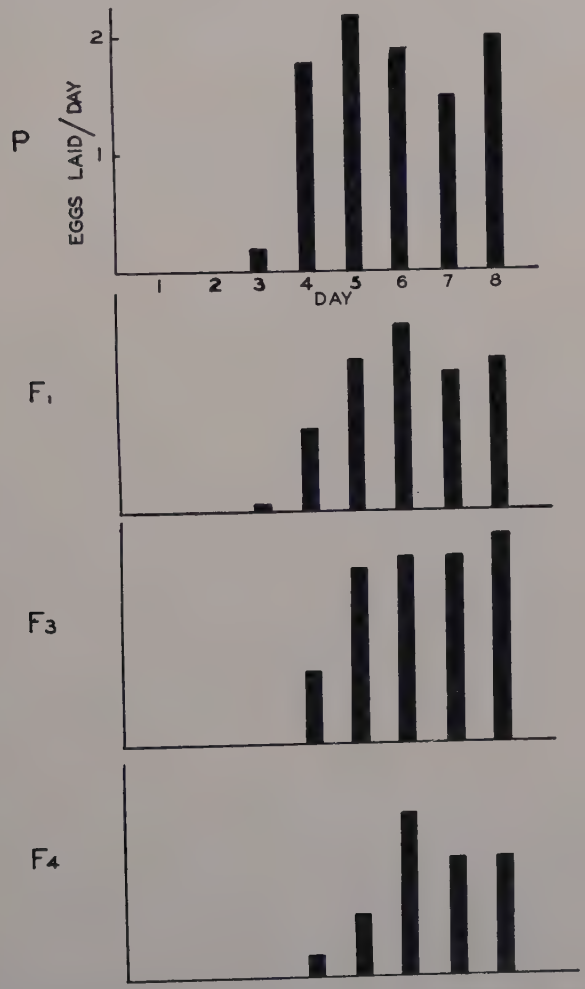

FIGURE 10. Similar to FIGURE 9 but illustrating data for a young orthoclone. Here, age of maturation and maximal egg production are retarded.

production is achieved earlier. In the terminal F_4 generation egg production is much reduced and the adult rotifer is much smaller than normal.

Quite the opposite occurs in a young orthoclone (FIGURE 10). As longevity increases in successive generations, there is a steady shift in time of onset of egg production to older ages. Finally, the rate of egg production is reduced and full grown rotifers are substantially larger than normal.

All of these observations may be summarized as follows:

(1) Age of the mother conditions longevity of the offspring;
(2) The aging factor is extragenic;
(3) The aging factor appears at the time of cessation of growth;
(4) Accelerated aging is accompanied by accelerated growth but decreased maximal size;
(5) Retarded aging is accompanied by retarded growth but increased maximal size.

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INFLUENCE OF MATERNAL AGE ON PENETRANCE OF "EYELIDS OPEN" IN NEWBORN MICE*

By Theodore S. Hauschka and Elizabeth Brown
Institute for Cancer Research and the Lankenau Hospital Research Institute, Philadelphia, Pa.

Open eyelids at birth have been found associated with the pleiotropic syndromes of various genotypes in the mouse, such as congenital hydrocephalus,[1] harelip,[2] and myelencephalic blebs.[3] The eye-anomaly has also been produced as a phenocopy by X-irradiation (300r) between the ninth and eleventh days of embryonic development.[4] In the present report, the trait symbolized as *eo* is similar to that described by Loeffler[5, 6] in 1932. Affected animals are born with one or both eyes open, whereas in normal infants eyelids remain closed for about two weeks after birth. The abnormal condition results from failure of the embryonic lids to fuse on the 16th day of foetal life, and is controlled by an autosomal recessive gene under the influence of several modifiers. Normal overlapping was gradually reduced, but not eliminated, by prolonged plus-selection. A pronounced maternal age effect on penetrance was investigated in two inbred strains and their sublines.

Stocks and Procedures

During the past six years, "eyelids open" was encountered in four of the mouse strains kept at this laboratory: DBA, A/St, A/He, and C3H/St. Selection for and against *eo* was carried out primarily in C3H/St, obtained from Doctor Strong in March 1950. In our second generation of this stock (corresponding to Strong's F77), *eo* occurred spontaneously and uncomplicated by other defects. Most matings were between brother and sister, except for a few backcrosses of daughters to fathers. The matings were set up at the time of weaning (unless deliberately postponed), and each cage contained only a single pair of mice which remained together during their entire reproductive life. Intervals between successive litters were thus kept quite constant, assuring close correlation between parity and maternal age. Diet and other details of maintenance were uniform throughout the period covered by the data. Litters were inspected and classified on the day of birth, and records were kept of sex of affected infants, asymmetry in manifestation of *eo*, and post-natal mortality, up to weaning age.

Observations

(I) *Results of selection and sex-difference in manifestation.* A newborn A/He mouse with open eyelids is pictured in FIGURE 1 (A). This animal also has a cleft palate (*hp*), which in Reed's data[2] was significantly associated with the eye-anomaly. In our A/He material 1096 mice were analyzed for the incidence of both harelip (*hp*) and eyelids open (*eo*) at birth: 40 were *hp*, *eo*; 125 *eo*; 82 *hp*; and 849 normal. Expectation for random coincidence of *hp* and *eo* is 37, which does not differ significantly from the actual 40 obtained.

* Supported in part by an institutional grant from the American Cancer Society.

FIGURE 1. (A). Newborn A/He male mouse showing eyelids open (*eo*) and cleft palate (*hp*) which, in our stock, did not coincide significantly.
(B). Haemorrhagic condition associated with *eo*. Corneal and other eye damage often persists through adult life.

TABLE 1
INCIDENCE OF "EYELIDS OPEN" AT BIRTH (1989 CASES) IN SIX INBRED STRAINS AND SUBLINES OF MICE

Strain	Selection	Number of matings	Total litters	Total ♀♀ born	Total ♂♂ born	Total ♀♀ affected	Total ♂♂ affected	Total per cent incidence
BAD	None	72	356	1134	1130	2	2	0.2
A/St	None	30	95	271	295	3	6	1.6
A/He	+, 2 Gens.	218	824	2345	2225	124	275	8.7
C3H/St, I	+, 7 Gens.	122	479	1506	1554	458	818	41.7
C3H/St, II	+, 8 Gens.	50	208	682	671	107	194	22.2
C3H/St, III	−, 6 Gens.	19	90	267	289	0	0	0

FIGURE 1 (B) depicts the inflammatory and haemorrhagic symptoms characteristic of the eye defect. In the absence of regular lid movements, the infant's eye is exposed to injuries, and permanent damage—especially to the cornea—is recognizable in most of the affected animals throughout life.

Incidence of *eo* in 12,369 mice of three inbred strains and their sublines has been summarized in TABLE 1. The trait was rare in DBA and A/St. Plus-selection was attempted in A/He, but was abandoned after two generations because of small litter-size, cannibalism, and nonviability of harelip individuals. In the C3H/St strain, analysis was not complicated by other genetic defects, litter-size was relatively large, and viability of the *eo* animals was good. Hence the investigation was concentrated on this strain.

Three sublines were derived from one litter of a brother-sister pair received from Doctor Strong in March 1950 (FIGURE 2). Sublines C3H/St I and II have been plus-selected for *eo* over eight generations. Counter-selection has completely eliminated *eo* from subline III.

Increase in penetrance due to selection, a pronounced sex-difference in mani-

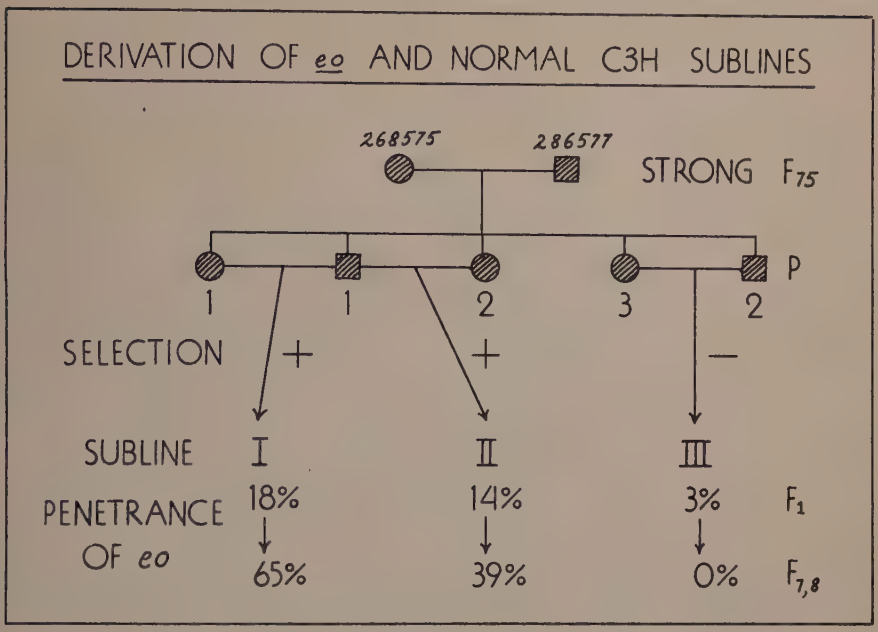

FIGURE 2.

TABLE 2

SEX-DIFFERENCE IN MANIFESTATION OF "EYELIDS OPEN" AND EFFECT OF SELECTION ON INCIDENCE AND DEGREE OF EXPRESSION IN C3H/ST, SUBLINE I

Generation	Total ♀♀	Total ♂♂	Per cent affected ♀♀	Per cent affected ♂♂	Expression in affected animals ♀♀ % Left	♀♀ % Right	♀♀ % Bil.	♂♂ % Left.	♂♂ % Right	♂♂ % Bil.
P-F_3	460	533	16.8 ± 1.8	32.5 ± 2.0	34	35	31	43	23	34
F_4-F_7	1046	1021	36.4 ± 1.4	63.3 ± 1.5	33	24	43	28	17	55

festation, and a decrease in the asymmetry of expression are evident from TABLE 2. For the incidence of *eo* in the early, as compared with the later generations, the difference divided by its standard error (D/SE) is 14.0, a highly significant value. For the sex-difference, D/SE = 13.1. For the increased frequency of bilateral *eo* resulting from plus-selection, D/SE = 5.5; and for the difference between unilateral left *versus* right *eo* in males, D/SE = 7.4.

The effect of selection on penetrance of *eo* in C3H/St I has been plotted separately for males and females in FIGURE 3. The graph is based on 81 sib-matings in all of which both parents were phenotypically *eo*, hence homozygous for the principal recessive gene, but probably heterozygous for parts of the modifying complex. These animals produced 312 litters totaling 948 males and 916 females, all inspected at birth. The considerable sex-difference persisted

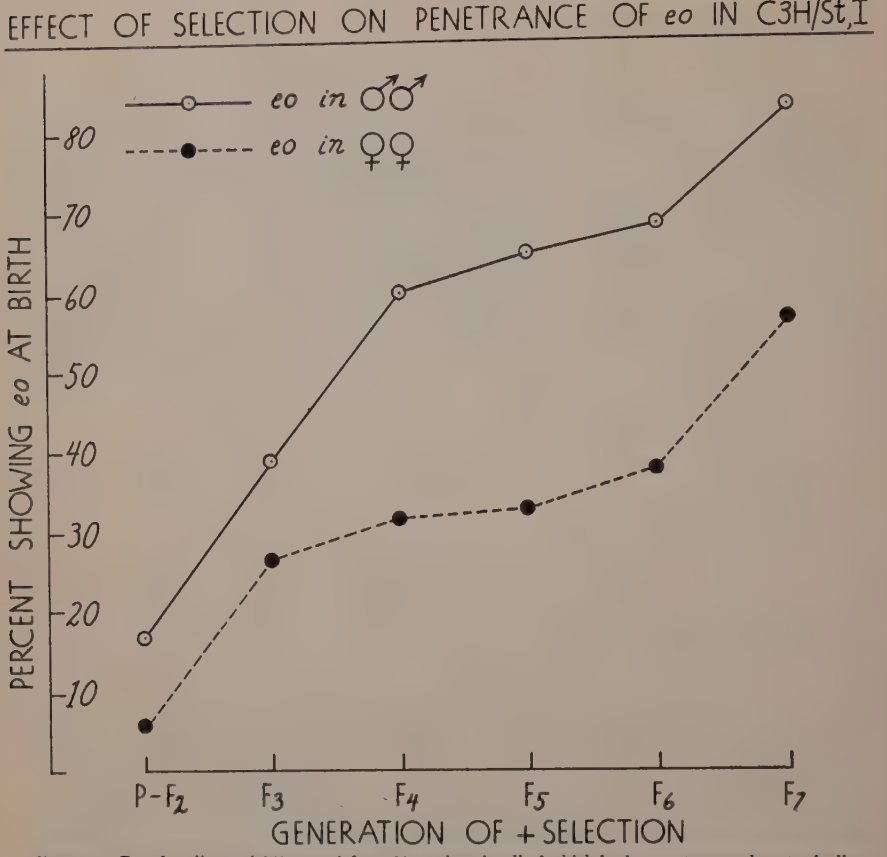

FIGURE 3. Based on litters (1864 yonng) from 81 matings in all of which both parents were phenotypically *eo*.

through seven generations. In this respect, our results differ from those of Loeffler,[6] in whose stock continued pairing of affected mice led to a disappearance of the sex-difference after only three generations. Since Loeffler was able to recover the sex-difference by counter-selection, he postulated X-linkage of a minus-modifier. Sex-linkage is not indicated by our data for the following reasons: In the absence of differential pre-natal mortality indicated by the normal sex-ratio at birth (TABLE 3), plus-selection should gradually eliminate

TABLE 3

RATIO OF *eo*♂♂:*eo*♀♀ IN FOUR TYPES OF MATINGS (C3H/St)

Type of mating	Number of matings	Total litters	Total young	Sex-ratio at birth	Ratio of % *eo* ♂: % *eo* ♀
+ ♀ x + ♂	16	73	496	51.4%	1.72
eo ♀ x + ♂	33	144	920	50.9%	1.64
+ ♀ x *eo* ♂	22	96	609	50.6%	1.76
eo ♀ x *eo* ♂	81	312	1864	50.8%	1.71

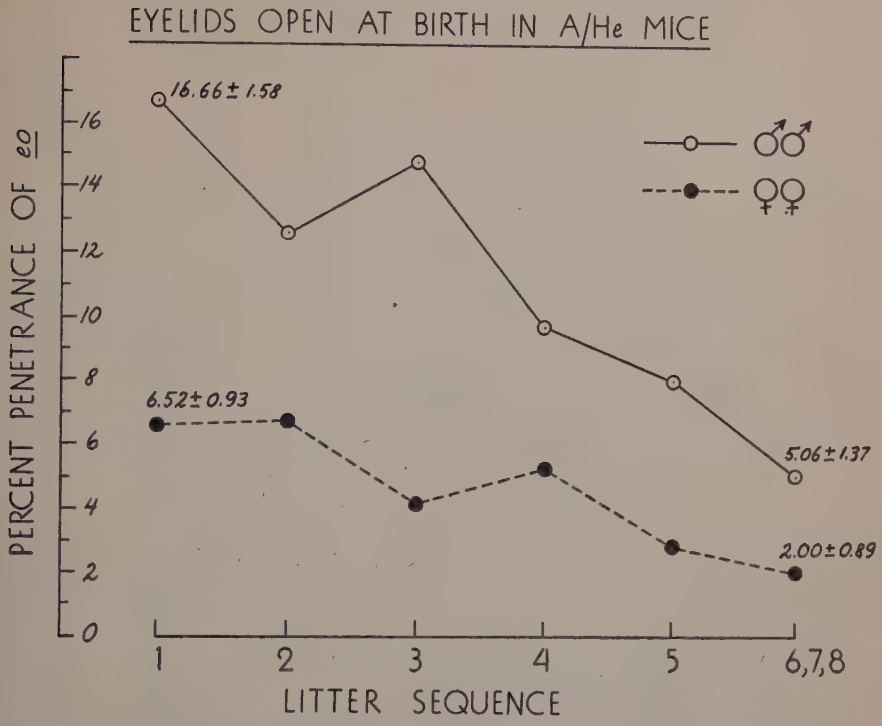

FIGURE 4. Based on a total of 4570 A/He mice, 399 of which were *eo* at birth. For the difference between first and last litters, D/SE is 5.5 in males, and 3.6 in females.

differences due to X-linkage, but failed to do so. Since reciprocal matings between affected and normal (TABLE 3) resulted in practically identical ratios of about 1.7 *eo* sons per *eo* daughter, Y-linkage of a modifier can also be ruled out. The higher frequency of *eo* in males may therefore be tentatively ascribed to a maternal influence.

(II) *Maternal age effects on penetrance of* eo. In the A/He strain, penetrance of *eo* declined gradually with litter sequence in both males and females (FIGURE 4). For the difference in per cent incidence between first and last litters, D/SE is 5.5 in the males and 3.6 in the females. Mean age of the mothers at first litter was 11.64 ± 0.45 weeks (standard deviation 3.15), and at fifth litter 35.28 ± 0.76 weeks (standard deviation 5.40). The latter value differs from the younger mean age of C3H/St mothers at fifth litter (TABLE 5), and suggests a somewhat slower reproductive cycle and less frequent conception at post-partum oestrus or greater delay in uterine implantation in the A/He stock.

The gradual decline of *eo* incidence in the offspring with mother's age (FIGURE 4) parallels the behavior of most other traits for which parental age-influences have been reported, and which show, either a continuous upward or downward slope with litter seriation.

A different, and rather unique, situation was encountered in C3H/St (FIGURE 5). Here, per cent *eo* shows a rise between first and second litters, followed by a

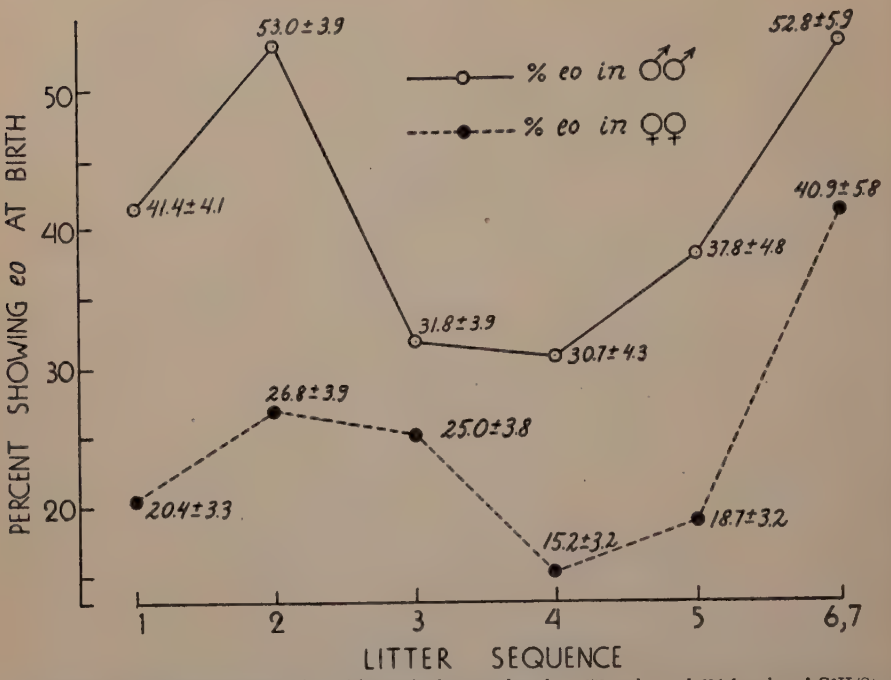

FIGURE 5. Per cent penetrance of *eo* with standard errors, based on 670 males and 626 females of C3H/St, subline I, born during the first four generations of plus-selection for *eo*.

TABLE 4

STATISTICAL COMPARISON BETWEEN PER CENT PENETRANCE OF "EYELIDS OPEN" IN RELATION TO PARITY IN C3H/ST, SUBLINE I*

Litters compared	Males		Females	
	Per cent difference in penetrance	D/SE	Per cent difference in penetrance	D/SE
1 and 2	11.6 ± 5.6	2.1	6.4 ± 5.1	1.3
2 and 4	22.3 ± 5.8	3.8	11.6 ± 5.0	2.3
4 and 6, 7	22.1 ± 7.3	3.0	25.7 ± 6.6	3.9

* These data refer to FIGURE 5.

sharp decline toward the fourth litter, and an equally steep increase between the fifth and last litters. Statistical analysis for these significant fluctuations is given in TABLE 4. Both sexes showed the same upward and downward trends throughout parity. A possible connection between the striking terminal increase in *eo* and hormonal changes accompanying incipient breast tumors in C3H/St mothers is implicated by comparison with the A/He data. Mean age at tumor appearance in C3H/St coincides with mean age at sixth

TABLE 5

AGE OF MOTHER AT BIRTH OF SUCCESSIVE LITTERS IN C3H/St, SUBLINE I*

Litter	Mean age of mother in weeks	Standard error	Standard deviation
First	11.06	± 0.46	3.26
Second	16.28	± 0.44	3.16
Third	19.98	± 0.45	3.24
Fourth	23.50	± 0.39	2.77
Fifth	27.22†	± 0.44	3.11

* The mean ages are based on the same material as FIGURE 5.
† Mean age at fifth litter approaches mean age at appearance of spontaneous breast tumors.

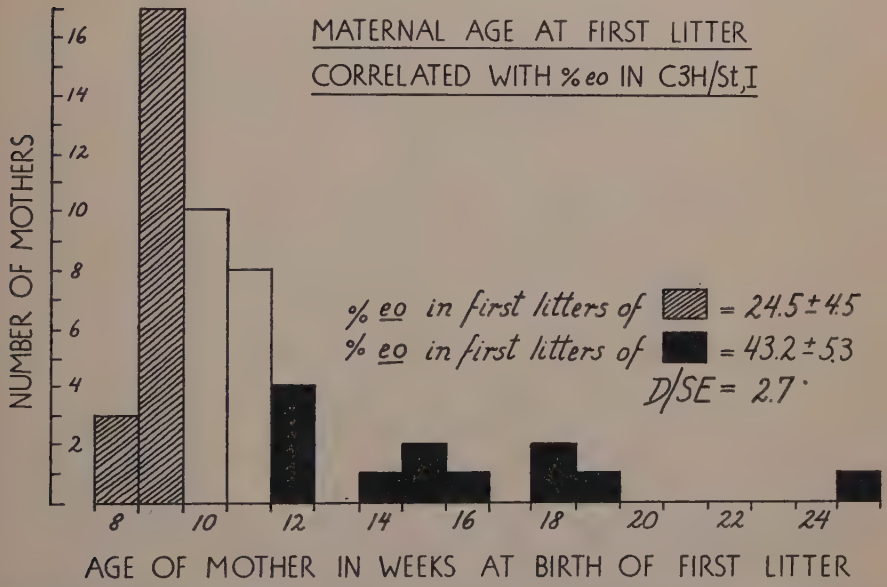

FIGURE 6. Based on 50 first litters of C3H/St, subline I, generations P–F_4 inclusive. The 20 youngest mothers had 23 *eo* offspring in a total of 94; the 12 oldest females produced 88 first litter offspring, 38 of which were *eo*.

and seventh litters, while tumors in the A/He breeding females occur much later (see discussion).

Although our mating system assured close correlation between litter sequence and maternal age (TABLE 5), it was, nevertheless, of interest to ascertain the role of mother's age uncomplicated by parity. In FIGURE 6, per cent *eo* in first litters of youngest mothers is compared with incidence in first litters of older females, some of which were deliberately allowed to age as virgins before being mated to their younger brothers, while others were slow to conceive. Since the older females produced significantly more offspring with the eye-anomaly, it may be concluded that maternal aging is primarily responsible for the observed shifts in *eo* penetrance with parity.

In FIGURE 7 the litter-seriation phenomenon is considered at three distinct stages of plus-selection for *eo*, *i.e.*, three levels of over-all *eo* frequency. Curves

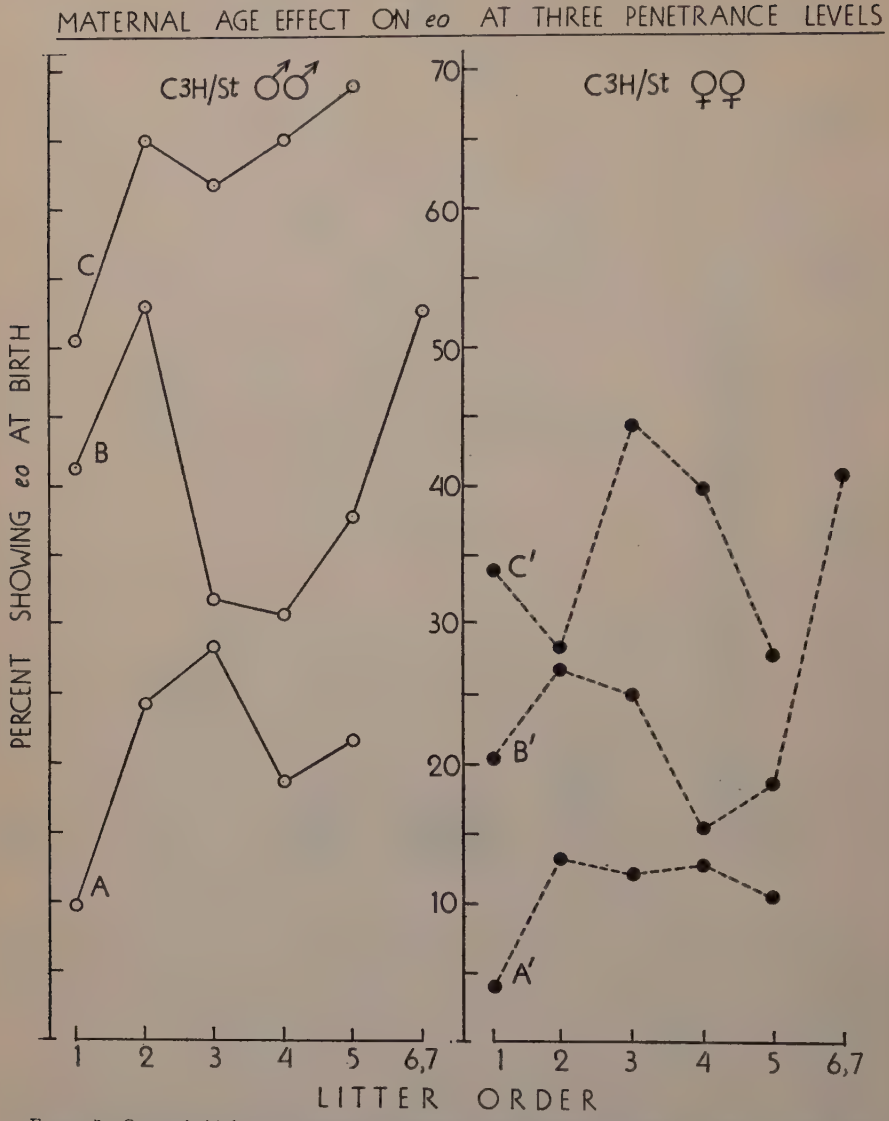

FIGURE 7. Curves A, A′ show *eo* penetrance in 380 males and 377 females of C3H/St, subline II, generations 1–4; B, B′ represent *eo* in 670 males and 626 females of C3H/St, subline I, generations 1–4; C, C′ are based on frequency of the eye-anomaly in 556 males and 551 females of C3H/St, subline I, generations 5–7.

A and A′ refer to the first four generations of C3H/St subline II; B and B′ represent the data for the first four generations of C3H/St subline I; C and C′ are based on generations 5 to 7 in subline I, where *eo* was most frequent. It is evident that males are the more responsive to maternal age effects and react similarly on all three levels of genic concentration. Female offspring behave in a less consistent fashion, except in curve B′, which follows all the trends of

the corresponding curve B for the male sibs. These results suggest threshold values for the interaction between maternal factors and a sensitive system of modifiers in the filial genotype. The maternal influence is most striking in the middle range of *eo* frequency.

Discussion

All the mammalian phenotypes for which significant fluctuations with maternal age are on record have one feature in common: they are controlled by genetically complex multifactorial and modifying mechanisms.[2, 5-19] Asymmetry in expression[2, 3, 5-7] and sex-differences in manifestation[2, 5, 6, 15] are, also, frequently involved.

The age-reactive traits include not only anomalies recognizable at, or soon after, birth (such as open eyelids, harelip, polydactylism, ear defects), but also inherited properties which do not become manifest until late adult life. Most notable among the latter is the positive correlation between mother's age (A and C3H strains) and incidence of spontaneous mammary tumors in the progeny.[11] This increase may, in part, result from a higher virus titer in the milk of older mothers. Leukemia in (Stoli × C58) BC mice, on the other hand, tended in the opposite direction.[12] Increasing parturition age progressively delayed the appearance of spontaneous leukemia in the offspring without influencing other causes of death. "The oldest mothers virtually eliminated leukemia, whatever the genetic tendencies of the family."

Penetrance of a filial trait can, thus, either increase or decrease with maternal age. One and the same character might show either an upward or downward trend, or no effect at all, depending on the genetic environment in which it is studied, as was observed by Strong[13] for fibrosarcoma latent periods in different mouse stocks.

The simplest explanation for the difference in reactivity of "eyelids open" to maternal age in the A/He and C3H/St strains would be that we are dealing with two different major genes, or at least two distinct modifying complexes. The same gene could, however, function differently against different backgrounds. A case in point is "white eye" in *Habrobracon*.[20] In most crosses, incidence of this trait was unaffected by the age of the mother wasp. When combined with "attenuated antennae," with which it showed linkage and which responded to mother's age, "white eye" now became significantly more frequent with successive batches of eggs, while "attenuated" showed a decreasing incidence in the same material.

On the assumption that "eyelids open" in the A/He and C3H/St mice is controlled by the same major gene (*eo*) and similar modifiers, the gradual downward trend with maternal age in A/He (FIGURE 4) and the U-shaped curve for C3H/St (FIGURE 5) may reflect hormonal differences associated with mammary tumor development in these two strains. Both strains harbor the Bittner milk-factor which, in conjunction with genotype and hormonal influence,[11] produces mammary tumors in 83 per cent ± 3.7 of the A/He breeding females and in 94 per cent ± 3.3 in the females of C3H/St, subline I, under our conditions of maintenance. In C3H/St, however, the tumors appeared at a mean age approximating sixth and seventh litters (*i.e.*, 30.4 ± 0.8 weeks; standard deviation 5.6),

while A/He mothers attained a mean age of 48.1 ± 1.4 weeks (standard deviation 12.6) before developing breast cancers. The steep upswing in penetrance of *eo* in the later litters of C3H/St (FIGURE 5) may thus be an expression of humoral changes concomitant with the genesis of malignant mammary tissue. This question could be tested experimentally through foster-nursing or transplantation of C3H/St, subline I, ova into a milk-factor-free strain, thereby establishing an *eo* stock with low tumor incidence.

Further indications that the age-effect on *eo* is exerted through interaction of filial genotype and maternal hormone balance are to be seen in the considerable sex-difference in reactivity of the embryos, and in the threshold aspects of the response which is most pronounced in the middle range of penetrance (FIGURE 7).

For his extensive data on litter seriation phenomena in the susceptibility of mice to methylcholanthrene-induced fibrosarcoma,[13-17] Strong has favored two alternative interpretations: "Do hormones fluctuate in the female body with advancing litter frequency, pass the placental barrier and influence the subsequent physiology of the offspring at least in relation to malignancy and perhaps other characteristics as well? Or are we to conclude that maturation phenomena in cytoplasm (perhaps the mitochondria or other constituents) are responsible for this transmission from mother to offspring which apparently is not through the genes?"[15]

The seemingly convincing alibi of the genes nevertheless deserves some scrutiny. The fact that the amount of crossing-over may change with the age of the female was first observed by Bridges in linkage studies on the second chromosome of *Drosophila melanogaster*.[21, 22] Age effects on recombination percentages are, by now, a generally recognized variable to be reckoned with both in the fruitfly and in the wasp *Habrobracon*.[20, 23] The only information on this point pertaining to the mouse was reported by Crew and Koller[24] who found a higher chiasma frequency in a six weeks old male than in several older specimens. Since it is evident from a comparison of our sublines I and II of C3H/St that these stocks, despite prolonged sib-matings, are not entirely homozygous for the plus and minus modifiers of *eo*, fluctuations in crossing-over with parental (either paternal or maternal) age would bring about differences in genetic composition of young from successive litters, and thus account for the observed differences in the penetrance of the major gene, *eo*. Prolonged plus-selection, then, should favor homozygosis of modifiers and lead to a state in which the *eo* complex approaches a single hereditary unit in its behavior. On this level, the maternal age influence could no longer manifest itself.

Our data for *eo* permit no first choice among the proposed interpretations. Critical experiments to decide this issue cannot, as yet, be performed, since pertinent knowledge of hormonal and cytological age-changes in the mouse is lacking.

Summary

Penetrance of "eyelids open at birth," controlled by a recessive gene (*eo*) and modifiers, and showing a pronounced sex-difference, is subject to maternal

age effects which differ in two inbred strains of mice. Changes in hormonal or cytoplasmic factors, or in crossing-over with parental age, are suggested as tentative explanations for the significant shifts in manifestation of *eo* with litter sequence, but the data permit no decision between these three alternatives.

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THE EFFECTS OF MERISTEM AGING ON THE MORPHOLOGY AND BEHAVIOR OF FRONDS IN *LEMNA MINOR*

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Introduction

The process of aging in plants differs in certain respects from the analogous process in animals. In most animals, the development of the organs takes place at about the same time; and all the organs mature, grow old, and die together. The plant is different in that its cells mature successively. The oldest cells are at the junction of root and shoot. The cells are progressively younger in time as one moves towards root or shoot apex, and at the apex itself the cells remain permanently meristematic, adding new cells to the existing plant body. It is therefore impossible to speak of the age of a shoot as a whole. Every horizontal layer of the shoot is younger than the layers below it and older than the layers above it.

This adds complications to the study of aging in plants, and makes it necessary to introduce the concept of physiological aging. Can we assume, for instance, that successive leaves on a shoot are alike physiologically when they reach the same time age? Does the meristem which produces the leaves remain as young physiologically as it does in time? Or do physiological changes occur in the meristem which are analogous to those occurring in other parts of the plant, *i.e.*, changes which are summarized by the term "aging"? There is as yet no general agreement on this question.

It would appear that there is plenty of evidence for the view that the meristem does age. There is a progressive change from node to node in the sizes, shapes, and physiological characteristics of the leaves of many plants. Such changes might be regarded as symptoms of meristem aging. It is, however, possible that these changes are due to concurrent changes in the environment. A leaf produced by a six-weeks-old plant may develop under conditions of light, temperature, day-length, *etc.*, which might be very different from those under which a leaf produced by a one-week-old plant developed. And, indeed, the changes in leaf size or shape in a number of plants have been satisfactorily explained in this way. In a number of other plants, including *Ipomoea*, which we used in our own work, they cannot be explained by any observable change in the environment.[2] It is notoriously difficult, however, to control the environment of a potted plant. Even if it could be done adequately, the meristem of a six-weeks-old plant might nevertheless be growing in a very different *internal* environment from that in which it was growing when the plant was one week old. The root/shoot ratio has changed, the meristem is now a great deal further away from the root system, and there are a great many more leaves between it and the root. Because of these complications, we have found it necessary to investigate the problem of aging at the meristem by means of a plant whose environment *can* be controlled adequately without difficulty and whose morphology remains approximately constant.

Lemna minor satisfies these requirements. It is a water plant which can be grown in a simple inorganic nutrient solution and under artificial light. It reproduces rapidly by vegetative means to give uniform clones. It is important for this discussion to understand the method of vegetative reproduction in *Lemna minor*. The entire plant body consists of a flattened, roughly elliptical frond, about 10 mm² in area, which is shown diagrammatically in FIGURE 1 (surface view) and FIGURE 2 (cross section). The figures show that daughter fronds arise on either side of the node, each at first enclosed in its own pocket in the mother frond tissue. They are first visible under the microscope

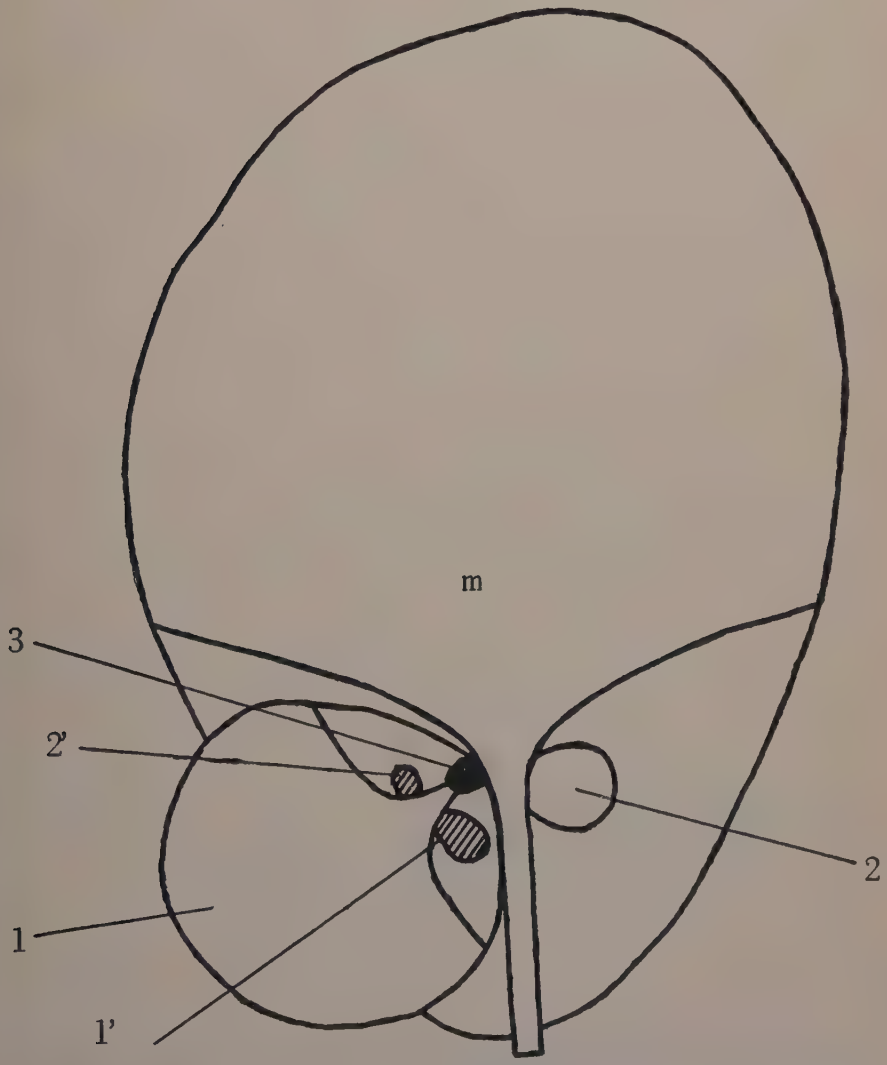

FIGURE 1. Diagram of surface view of *Lemna minor*, with flaps of pockets removed. m: mother frond; 1, 2, 3,: first, second and third daughter fronds; 1', 2': first and second granddaughter fronds. (×25 approx.)

as small, rounded, meristematic knobs, which grow, first by cell division alone, and later by cell division at the base and cell expansion elsewhere. Very early in the development of a daughter front, a knob of meristematic tissue appears on its dorsal side, close to the end which is in contact with the mother frond. This knob of tissue (which is the next daughter frond) does not develop any further until the first daughter frond is fully grown. At this time a few cells at the base of the frond elongate to many times their original length, forming a long stipe which separates the fully-grown daughter frond from the embryonic daughter frond and from the mother frond. Eventually the stipe breaks and the first daughter frond begins an independent existence. As soon as the first daughter frond has broken away from its mother frond, the embryonic frond which it has left behind begins to develop in its turn into the third daughter frond, the second daughter frond having in the meantime developed in the pocket on the other side of the mother frond.

Our material, therefore, consists of a piece of leaflike tissue with a single root, and two meristematic regions with young primordia in close contact with the leaflike tissue. The breaking away of fully grown daughter fronds results in a constant amount of meristematic and adult tissue in the mother frond throughout the whole of its life. This plant is therefore excellent material for studying the effect of the age of the adult tissue on the characteristics of its vegetative offspring; for, in fronds grown in a constant environment, the age of the mother frond is the only factor which varies in the course of the development of successive daughter fronds.

Experiments and Their Results

The observations and experiments on *Lemna minor* reported in this paper fall into two groups. Firstly, those which were designed to investigate whether aging at the meristem occurs and to find whether the rate of aging could be altered experimentally; secondly, those which were designed to analyze the causes underlying aging at the meristem. The first group have given us a com-

FIGURE 2. Diagram of cross section of *Lemna minor*, through pockets. m: mother frond; u: upper surface; l: lower surface; 1,2,3,: first, second and third daughter fronds. (×30 approx.)

prehensive picture of the course of aging throughout the life of a frond and of its effect on the daughter fronds. The second group have helped us to dispose of many likely factors which have turned out *not* to be the causes of aging; but they have, as yet, given us no clear indication of what the causes might be.

For the purpose of these experiments, fronds of *Lemna* all belonging to the same clone are grown in a large volume of culture solution which is renewed twice a week and kept at controlled temperature and pH; the fronds are illuminated with "daylight" fluorescent tubes at controlled intensity. It was found that morphologically equivalent fronds grown under identical external conditions have almost identical lengths of life. In full culture solution at 25°C and under 400 f.c. light for 14 hours a day, the length of life is about seven weeks. This is the time elapsed between the emergence of the frond under observation from the pocket of its mother frond, and the death of the frond as shown by loss of chlorophyll and collapse of the cells. In the course of its life a frond produces about a dozen daughter fronds under these conditions. The death of the fronds after a definite time interval, in a constant and favorable environment, can be ascribed only to internal causes. These internal causes can collectively be described by the term "physiological aging."

Our earliest experiments, then, established that fronds of *Lemna* grow old and die, even in a constant environment.[1] This raised the question of what effect, if any, the aging of the mother frond would have on its daughter fronds. To investigate such possible effects, the area, cell size, and life histories were examined of all the daughter fronds produced during the life of mother fronds growing in various constant environments. The data showed that successive daughter fronds produced by the same mother frond are not all alike, even though they are produced in the same position and in exactly the same external environment. The areas of successive daughter fronds when fully grown becomes progressively smaller, the older the mother frond. A typical set of results is entered in TABLE 1.

This progressive decrease in area is fairly regular. It takes place in such a way that when the area of the daughter fronds is plotted against the age of the mother frond when the daughter frond first became visible, the points lie on a line which cuts the abscissa near the point which indicates the time of death of the mother frond. Late daughter fronds are smaller than early daughter fronds because they have fewer cells. The cell *size* of early and late daughters is the same.

TABLE 1

Age of mother frond (days)	Area of daughter fronds (mm^2)	Age of mother frond (days)	Area of daughter fronds (mm^2)
3.5	9.9	28.9	7.0
5.6	9.7	33.2	6.2
9.0	7.9	36.9	5.5
11.8	8.2	41.3	5.4
15.1	7.6	44.4	4.1
18.4	6.9	51.2	3.9
22.2	6.0	55.8	2.5
25.1	6.7	60.3	1.6

It is possible to alter the length of life of the fronds by altering either the temperature[4] or the nitrogen level of the culture solution.[4, 3] An increase in temperature reduces, and a decrease in temperature increases, the length of life of the fronds. Similarly, nitrogen starvation increases the length of life of the fronds. Data illustrating the effects of temperature and nitrogen level on length of life are entered in TABLE 2.

These results raise a further question: How do such changes in length of life of mother fronds affect the area of the successive daughter fronds produced by them? We found that any treatment which alters the length of life of the mother fronds also alters the rate of diminution of area from daughter to daughter frond. This is illustrated, for example, by the effects of growing one set of fronds at 20°C and another at 30°C. This is shown graphically in FIGURE 3. The figure shows that raising the temperature by 10°C approximately halves the length of life of the mother fronds and at the same time doubles the slope of the line drawn through the points representing the area of successive daughter fronds. The figure shows also that, at both temperatures, this line cuts the abscissa close to the point of death of the mother fronds.

Two conclusions can be drawn from this correspondence between length of life of mother frond and rate of diminution of area of daughter fronds: (1) That area of daughter fronds can be used as a measure of the physiological age of the mother frond, and (2) that rate of diminution of area from daughter to daughter frond can be used as a measure of the rate of aging of the mother frond.

TABLE 2

EFFECTS OF TEMPERATURE AND NITROGEN LEVEL ON LENGTH OF LIFE OF *LEMNA MINOR*

Temperature (°C)	Length of life (days)	Nitrogen level (mgm/1)	Length of life (days)
20	73.4 ± 1.9	No added N	78.9 ± 2.3
30	40.4 ± 3.8	5.0	50.7 ± 1.4

Mother frond age not only affects daughter frond area; daughter fronds produced by an old mother frond also have shorter lives and fewer offspring than their sisters which were produced when the mother frond was younger.

These observations show that the physiological age of the mother frond is reflected in the morphology and behavior of its vegetative offspring. Let us next consider the life history of succeeding generations of fronds. It might be supposed that a late daughter frond at the beginning of its life is physiologically as old as the mother frond which produced it, and that its own progeny will be older still. The following observations, however, showed that this is not so.

It was shown above that a daughter frond produced by an old mother frond (*i.e.*, a late daughter) is considerably smaller than the mother frond which produced it. But the first daughter frond produced by this small daughter frond is itself not equally small or smaller, but considerably larger than the small frond which produced it. This increase in area of the first daughter frond, compared with its mother frond, continues from generation to generation until maximum area is restored. If one starts with a very small daughter

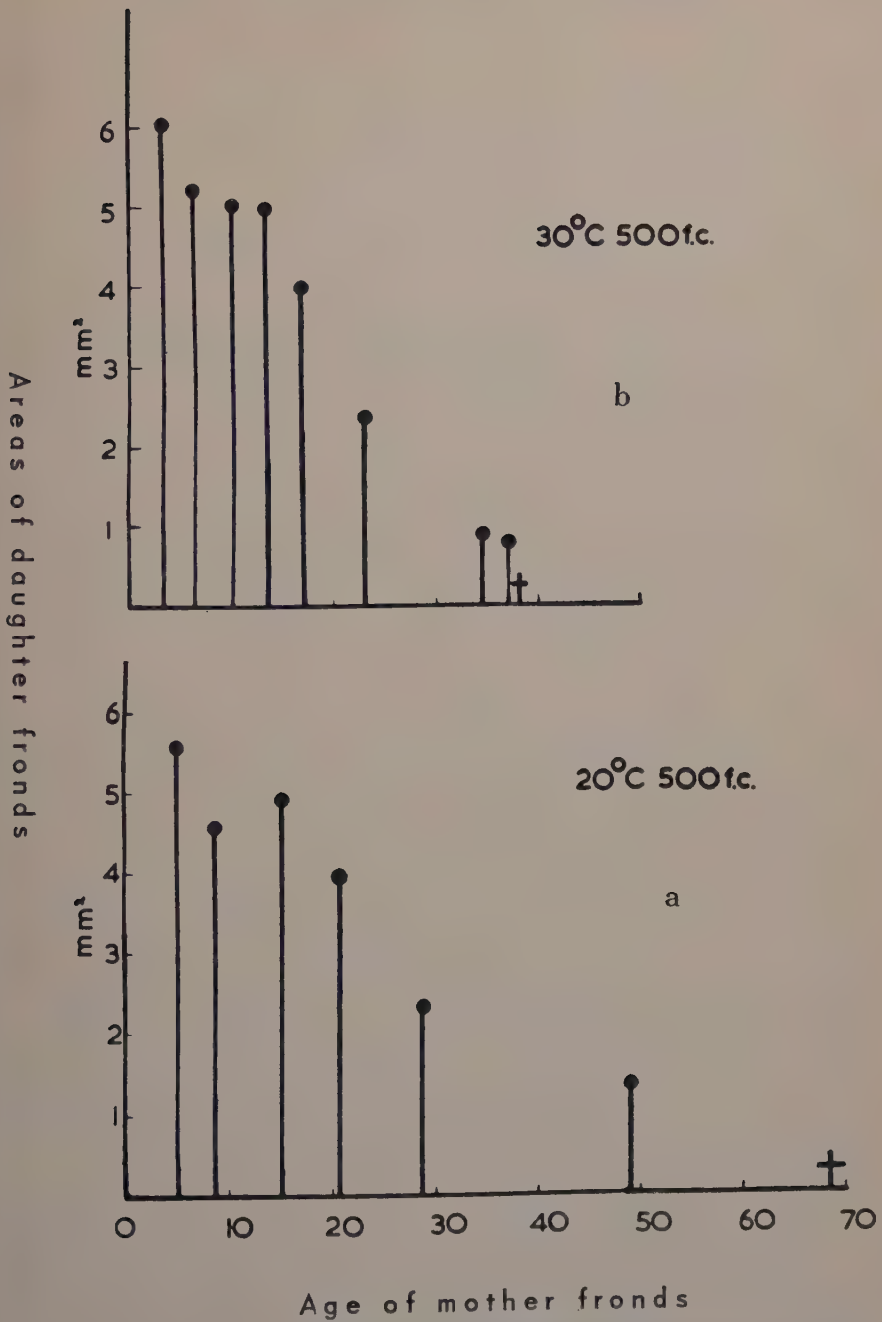

FIGURE 3. Area of daughter fronds (ordinates) plotted against age of mother frond when the daughter fronds appeared (abscissae). a: fronds grown at 20°C; b: fronds grown at 30°C; + represents time of death of mother fronds.

frond, it may take up to six generations to restore maximum area. This progressive increase in area of first fronds from generation to generation counteracts the progressive decrease in area of successive fronds of the same generation. The result is that the average frond area of a clone remains constant. Increase in area under constant conditions can be regarded as a symptom of rejuvenation at the meristem, just as decrease in area can be regarded as a symptom of aging.

The following is a summary of the course of aging and rejuvenation as it affects the meristems responsible for vegetative reproduction in a clone of *Lemna*.

Let us suppose we are starting a new clone with a young frond of maximum size. It produces daughter fronds throughout its life. Its first daughter is as large as itself, but each successive daughter it produces is smaller than its elder sister, *i.e.*, the meristems become old together with the mother frond. Each successive daughter frond now in its turn becomes a mother frond and produces daughters of its own, which are granddaughters of the original frond. The same thing happens; each granddaughter is smaller than its elder sister. But with this difference: the *first* granddaughter is larger than the daughter which produced it, the percentage difference being greater the smaller the daughter, *i.e.*, the older the original mother. Thus we have progressive aging of the meristems of successive daughters on any one frond, and progressive rejuvenation of the meristems from one generation of fronds to the next.

Discussion

It has proved very difficult to analyze the causes underlying these cycles of aging and rejuvenation. Aging is not a consequence of the production of daughter fronds: for, in the first place, the rate of production of daughter fronds can be changed without at the same time changing the rate of aging;[4, 5] and, in the second place, all daughter fronds can be removed as soon as they appear (a treatment which also reduces the total number of daughter fronds) without changing the length of life of the mother frond.[5] These observations show that aging takes place in the mother frond, whether daughter fronds are produced or not. The progressive reduction in the number of cells per daughter frond when they *are* produced must be regarded as due to a progressive change in the effect which the mother frond has upon the daughter frond primordia.

Numerous experiments have been carried out which were designed to investigate the nature of that effect. They involved excision of primordia at various stages of growth from mother fronds of various ages, and addition of various substances to the culture medium. These experiments have led to the tentative conclusion that young daughter fronds need some growth-promoting substance (other than major nutrients) from their mother fronds, if they are to reach maximum area; and that meristem aging consists in their not being able to obtain enough of this substance from the mother frond. This might be either because of a decline of this hypothetical substance in the mother frond as it gets older or because of an accumulation of some other substance which inhibits or destroys the growth promoting substance.

Experiments have shown that the hypothetical growth-promoting sub-

stance which becomes scarce in the course of aging is not auxin or adenine.[6] Recent evidence suggests that long life might be associated with low respiration and *vice versa*.[3] This possibility is now being investigated.

It remains to show how the phenomenon of rejuvenation can be accounted for by the hypothesis that aging of the meristem is caused by a decline in or inhibition of a necessary growth-promoting substance in the mother. We must suppose that a young primordium produced by an old mother manufactures the growth-promoting substance during its own growth to maturity, and that it makes more than it received from its old mother frond. It cannot, itself, benefit from what it makes, but its daughter fronds can; hence they become bigger than their mother frond. This idea is consistent with observations we have made on excised fronds. When daughter fronds of young mother fronds are severed from their mother fronds before the daughter fronds are fully grown, they go on living but never reach their full size. They produce daughter fronds, however, which are larger than the excised fronds themselves, and the increase in area continues from one generation to the next until maximum area is restored. We can suppose that by removing daughter fronds from young mother fronds before the daughter fronds are fully grown, we imitate artificially what happens naturally in old mother fronds: we cut off the supply of the growth promoting substance, and thus reduce the frond area of the daughter frond, but the daughter frond itself produces more growth promoting substance which it passes on to its own daughters, and these daughters can therefore reach a bigger area than their small mothers.

In the preceding paragraphs a hypothesis was put forward to account for the symptoms of aging and rejuvenation found in *Lemna*. This hypothesis is so far consistent with all the data, but needs experimental confirmation. The situation can be summarized briefly as follows: A clone of *Lemna* as a whole does not age. Individual fronds age and die, but rejuvenation takes place during vegetative reproduction, with the result that the *average* physiological age of the clone remains constant. It remains to be seen whether this is in any way unique to *Lemna* or whether a similar process occurs in other plants which habitually reproduce by vegetative means.

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PARENTAL AGE AND GERMINATIVE CHARACTERS OF THE SEEDS

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Many species of plants show no signs of aging in their growing points, while other species show distinct processes of aging. These processes may be very slow as, for example, in many species of *Bambus* and *Agave*, or in several types of palm trees, where it takes some years before the growing points show manifestations of aging. This aging becomes apparent by the diminishing size of leaves and, very often, by the fact that only flowers appear. The formation of flowers is suppressed by the leaves as long as new leaves are formed; that is, as long as the plant is comparatively young.

There exist, also, species of plants, the aging of which is not continuous but rhythmical. In these cases the individual plant shows inactivations and depressions of life activity which are, in all respects, similar to the beginning of the type of aging mentioned above. In the case of periodic aging, however, these depressions are only temporary. They are followed by a rejuvenation. We need only mention, as an example, the perennial plants of temperate regions with their annual period of inactivation during the winter. There may be some doubt as to whether these periodic inactivations are comparable to the inactivations which actually lead to death. But these doubts are removed, not only by investigations on the physiological similarity between the depression of life activity during the fall, and preparation for death, but also by the fact that, in the case of reversible inactivation, many parts of the plant; leaves, stems, *etc.*, indeed often all parts, except rhizomes or bulbs, actually do die. Thus the annual period of inactivation or rest is a phase of aging which leads in some parts to death, in others only to inactivation followed by rejuvenation.

In this type of plant, the surviving parts show a gradual decrease of life activity in the fall, and a gradual reactivation during the ensuing months. We know, definitely, that this periodic aging and rejuvenation is not merely induced by the fluctuations in the external factors, but is distinctly an autonomous process, which also goes on under constantly controlled, or laboratory conditions.

It is a surprising fact that the seeds ripening on these plants may continue to show, during their time of connection with the mother plant and during the following months (that is, during their rest period), the same inactivation and reactivation as the mother plant. This is clear, at any rate, with the seeds of several species. The consequence of this dependency is that a seed from an early flower (that is, a seed ripened during the spring or early summer) has quite different qualities compared with a seed from a later flower. These differences may be found, especially, when testing the germinative qualities. It is a well-known fact that the seeds of many plants show a period of dormancy during which no germination or only a very delayed germination, is possible.

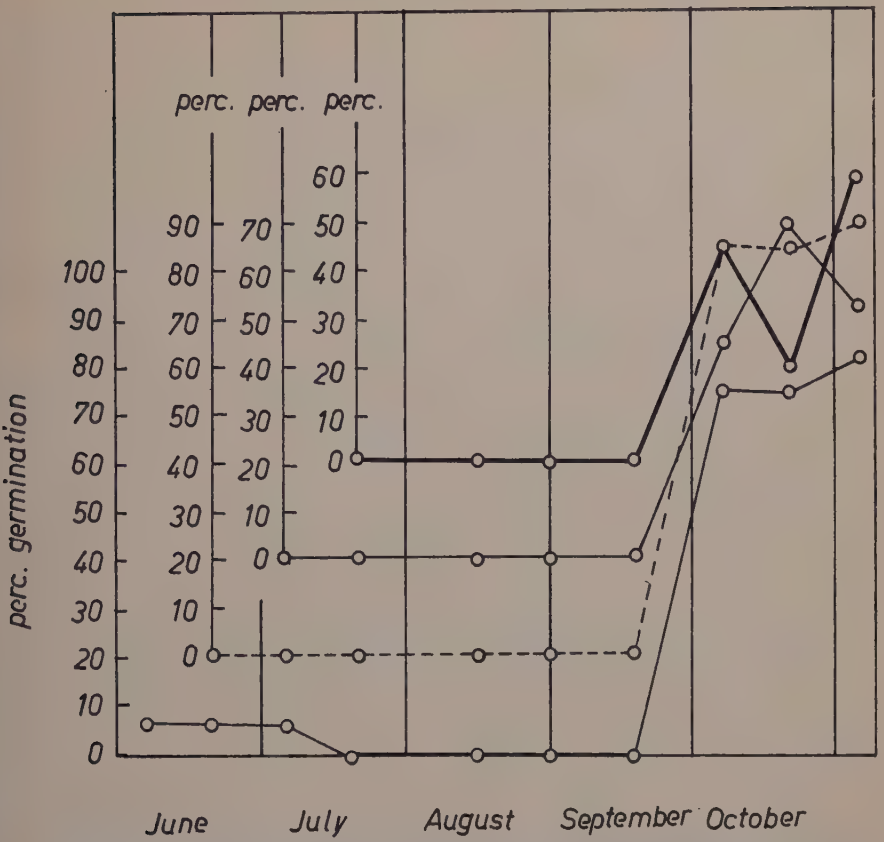

FIGURE 1. Seeds of *Fragaria vesca* showing percentage of germination at different times. The seeds were removed from the plant immediately after ripening. The time of ripening varied according to the time of flowering. The curves begin with the times of harvesting: June 6; June 19; July 4; July 17. In spite of the different times of ripening, the times of the end of dormancy correspond to each other in all series. That means the duration of after-ripening is long in the first set and shorter in the following ones. Moreover, the first set shows, at the beginning, a period of a certain degree of activity.

It takes a couple of months of after-ripening before the percentage of germinating seeds, in a given test, gradually approaches 100 per cent. Now, it becomes clear that this period of dormancy is, at any rate in several cases, nothing else but the annual period of decreased, or fully suppressed, life activity which characterizes the growing points and other embryonic tissues of the mother plant.

This identity is best demonstrated by an example, see FIGURE 1. Seeds of strawberry plants (*Fragaria vesca*) harvested after their ripening early in the year show, at first, a certain period of germinative capability that means a certain percentage of them will germinate if brought into a suitable substratum such as moist filter paper. Samples of the same seeds stored under constant conditions, but tested a couple of weeks later, are no longer capable of germinating. Later, however, these seeds are able to germinate (FIGURE 2). This

behavior corresponds exactly to the fluctuations in the life activity (formation of new leaves and growth) of the mother plants. Seeds of the same plants having ripened a little later and, therefore, been harvested a little later are at first dormant. The period of activation begins at the same moment as that of the early ripened seed. Thus, their rest period is shorter. Those seeds that ripened later show a still shorter rest period. Therefore these facts demonstrate that the seeds, in the moment of their dispersal from the mother plant, possess, at this time, the same "status of age." Thus the gradual breaking down of this dormancy is the same thing as the process of dormancy and rejuvenation in the buds of the mother plant. This means that the growing points of the embryos in the seeds have exactly the same physiological behavior as the growing points in the buds of the mother plant.

There is a surprising similarity between the rest period in the mother plant and the period of dormancy in the seeds. It is shown, in several species of plants, by comparing the course of the rest period in the rhizomes, *etc.*, with that of the dormancy in the seeds (FIGURE 3).

It must be taken into consideration, however, that not only these processes of periodic aging but also other internal factors may influence the germinative qualities of the seeds. Therefore the facts mentioned above do not hold for all species of plants.

The continuation of a physiological process from the adult plant to the offspring is astonishing as long as one believes this process to be connected with complicated interfering processes. But, apparently, the processes on which the periodicity of life activity is based, that is, of periodic aging and rejuvena-

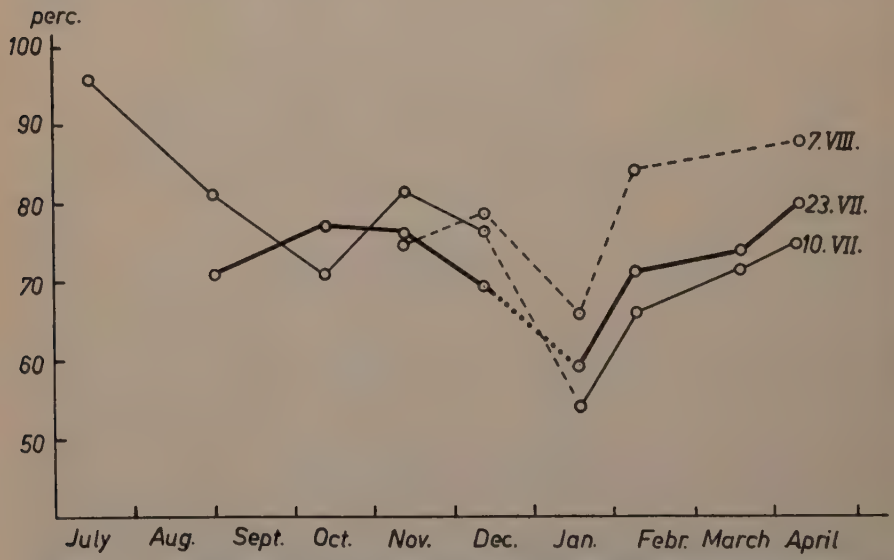

FIGURE 2. As in FIGURE 1 but another variety of *Fragaria vesca*. These sets show more clearly the existence of a period of activity immediately after ripening. Times of harvesting are shown at the right. From both figures it is clear that the degree of activity of the seeds at the time of harvesting always corresponds to the degree of activity of the mother plant at that moment. The lowest activity occurs during the fall and at the beginning of winter, but is somewhat different in the two varieties.

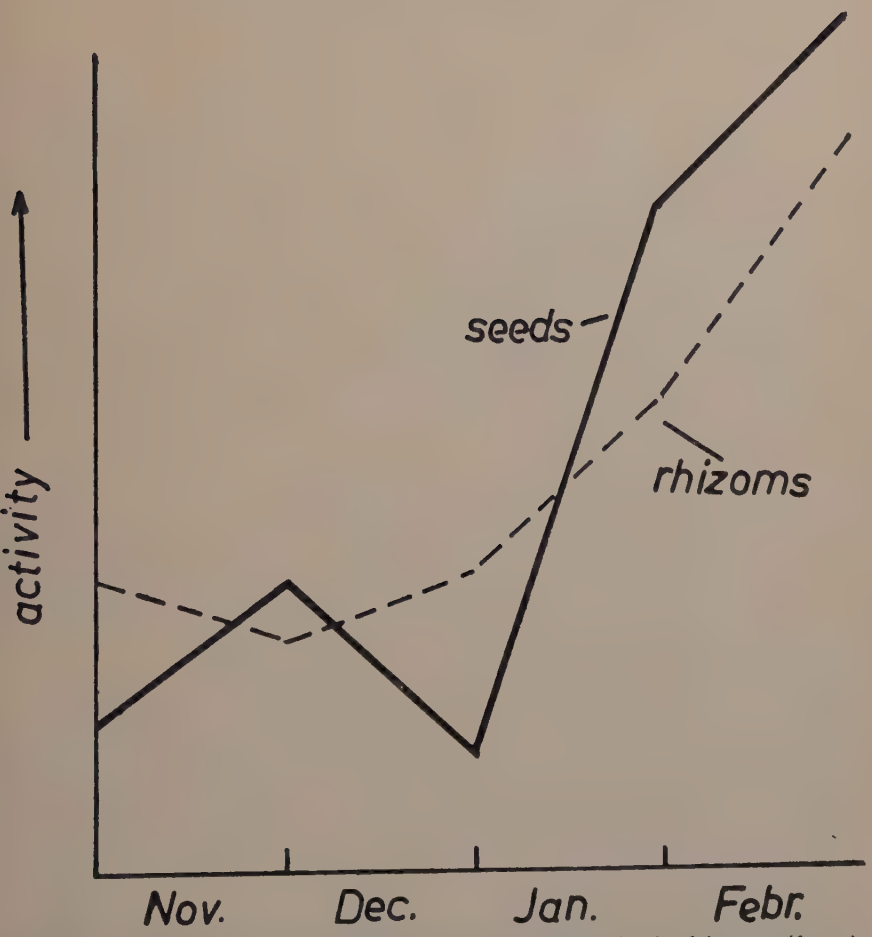

FIGURE 3. *Veronica officinalis*. Activity of seeds and rhizomes. The activity of seeds is measured by testing the percentage of germination; the activity of rhizomes by testing the rapidity of growth at different times of the rest period. Conditions were, of course, maintained constant at all times.

tion, are not, according to new investigations, biochemical, but biophysical in nature. The periodicity is interrelated with alterations in the colloidal status of the protoplasm, alterations which become apparent, for example, in fluctuations of the water binding capacity. Perhaps a continuation of these biophysical processes from one generation to the next is easier to understand than a continuation of biochemical processes from the mother plant into the seeds.

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THE AGING OF SEEDS AND MUTATION RATES*

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After reading a paper by Navashin[1] in which he reported finding more chromosomal abnormalities in roots from old than from young seeds of *Crepis*, it occurred to us that aging of seeds might also increase the mutation rate of gene mutations. Spontaneous gene mutations are extremely rare in *Datura stramonium*. Going back over our records from 1909 to 1934 we found only five gene mutations from selfing approximately 12,000 untreated plants. This is a mutation rate of ±0.042 per cent. From selfing 392 plants which had come from seeds which were 7½ to 9½ years old we had recorded five gene mutations showing morphological characters, which is a mutation rate of 1.27 per cent. This mutation rate from aged seed is about 30 times that recorded from young seeds.

We had discovered that mutations causing pollen abortion genes is several times as frequent as mutations which bring about recognizable morphological characters. A special study was made, therefore, of pollen abortion genes in cooperation with Cartledge.[2] Before detailing our results with pollen lethals, it will be well to say a few words about the work of Doctor Satina[3] on periclinal chimeras. By the differential increase of chromosome numbers through colchicine treatment, she was able to show that there are three germ layers in *D. stramonium* and that it is primarily the second germ layer which gives rise to the egg cells and pollen grains. Due to the rarity of mutations it is inconceivable that two cells of the second germ layer in the seed would be affected by the same mutation although several of the cells of this layer might have different mutations. As the plant develops from the seed the cell offspring of the individual second layer cells will form sectors in the adult plant. A sector heterozygous for a pollen lethal gene will, through reduction divisions in the flower bud, give rise to half of the pollen grains with the lethal which will abort and half which are normal pollen. Our practice is to examine microscopically the pollen of one flower (two flowers in some cases) from each plant and if 50 per cent of the pollen is bad to examine adjacent flowers in order to delimit the sector. Through pollen tests and tests of offspring from different capsules, we have had evidence of as many as five different sectors in a plant. This means that there were a minimum of five cells in the second layer which took part in the development of the adult plant. We, also, have evidence that sometimes only one cell of the second layer has contributed to the adult growth.

Table 1 gives the results of one-flower samples taken from over 5,000 plants which had grown from seeds of different age. It will be seen that the mutation rate increases with the number of years the seeds had been aged on the laboratory shelf. Doctor Cartledge believed he could distinguish, by differences in appearance, the pollen abortion due to a gene from that due to a chromosomal abnormality and called them gene types and chromosomal types.

* Contributions from the Department of Botany of Smith College. New Series No. 54.

TABLE 1
POLLEN ABORTION AND AGE OF SEED
(Summary, 1-flower samples)

Age of seed in years	Plants recorded	Pollen abortion types			Percentage
		Genes	Chromosomal	Total	
9–10	100	4	3	7	7.0
7–8	242	5	11	16	6.6
6–7	14	0	0	0	0.0
5–6	49	1	1	2	4.1
4–5	604	13	7	20	3.3
3–4	1289	10	9	19	1.5
2–3	911	4	2	6	0.7
1–2	1324	2	9	11	0.8
0–1	939	1	3	4	0.4
Totals	5472	40	45	85	—

Doctor Satina from cytological study of these two types showed that the distinction held in most cases, though there were a few exceptions. The chromosomal type suffers abortion of the grains at an early stage leaving the affected pollen shrivelled and empty. The gene type usually has the affected grains showing abnormalities of smaller size and defective contents.

The value of pollen abortion as an index of mutation rate over visible gene mutations is their greater frequency and the fact that the determinations can be made relatively rapidly in the first generation, whereas to secure visible gene mutants (which in *Datura* are almost exclusively recessives) a considerable number of offspring must be grown in the second generation.

Aging pollen was found to give an increase in pollen mutations in the hybrids with normal female parents.[4] In such hybrids no sectors would be expected and all the flowers were of the same type.

Both gene and chromosomal mutations have been induced by treatment of seeds with different types of radiation. There is no evidence, however, that such agents have been responsible for the spontaneous mutations in nature. Seeds of many plants have been known to retain their vitality when buried in the soil for many years and it occurred to us that such seeds aged under natural conditions might show an increased mutation rate.

Shortly after our initial experiments on effects of aging *Datura* seeds which had been stored on laboratory shelves, we are able to test seeds of *Datura* which had been buried in the soil. Mr. H. B. Derr, County Agent at Fairfax, Virginia, had excavated soil from his cellar in order to install a furnace. The excavated soil gave rise to a vigorous crop of Jimson Weeds (*Datura stramonium*). Through the cooperation of Mr. Derr we were able to secure several bags of soil from the unexcavated parts of the cellar. From this soil we obtained nearly 500 seedlings.[5] The evidence was good that they had remained buried in the cellar soil for at least 22 years. Using two-flower samples we found only eight pollen types, which is a mutation rate of 1.8. Such a mutation rate, if significant, is small in comparison with the rate from seeds aged in the laboratory.

The seeds from the cellar in Virginia were in very dry soil. Since in a cooperative study with Doctor L. V. Barton of the Thompson Institute[6] it was found that increased moisture content of seeds increased the mutation rate, it was felt that a different result might be found if we had been handling seeds which had been buried in moist soil.

An opportunity to test seeds stored in soil under more natural conditions arose in 1941. In 1902 the U. S. Department of Agriculture buried seeds of a number of species and tested their viability at five- and, later, 10-year intervals. We learned of this experiment after a sample of the seeds had been dug up in 1940 but too late to make a genetic test of the *Datura* seeds. The need of erecting a government building at the plot in Arlington where the seeds were buried enabled us to secure the Datura seeds in 1941. The seeds which had been buried 39 years gave over 90 per cent germination. Of the 356 plants secured from these buried seeds[7] no pollen abortion types were secured. So far as the tests of buried seeds offer evidence, it can be concluded that aging of itself is not responsible for increased mutations from old seeds. Other associated factors must be of influence. In the cooperative study with Doctor Barton[6] it was found that heat increased the mutation rate in *Datura* seeds and that increasing the moisture content of the treated seed induced a greater number of mutants. What influence heat and moisture had upon the mutation rate of laboratory stored seed is, as yet, undetermined.

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WRIGHT'S STUDIES OF EFFECTS OF MATERNAL AGE ON SPOTTING AND POLYDACTYLY IN THE GUINEA PIG

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In mammals, where early development takes place in an environment of maternal tissues, changing physiological conditions of the mother constitute a changing environment which can affect the outcome of a developmental process in the offspring, within the range of variation permitted by the hereditary constitution. For example, the age of the mother has been shown by the detailed studies of Wright[1, 2, 3] and Wright and Chase[4] to be a major factor, if not the most important factor, common to littermates in producing nongenetic variability in two different cases of inherited characters: spotting of coat and polydactyly in the guinea pig.

White spotting. The piebald pattern of pigmented and nonpigmented areas of the skin and coat depends primarily on a recessive Mendelian factor, *s*, with many minor genetic factors, with additive effects occurring in different strains.[4] Even in highly inbred strains, however, there is much variation in relative amounts of pigmented and nonpigmented areas. An intensive study of the correlations between percentage of white and environmental factors, such as season of birth, size of litter, and age of dam, revealed that the mother's age was a major factor, in a group of factors common to littermates, in producing nongenetic variability. This effect of maternal age is readily seen in TABLE 1.[1] It is evident that, with increasing age of dam, there is an increasing percentage of white in the offspring. The possible effect of age of sire was ruled out by mating males and females of widely differing ages. In these matings, age of dam was the deciding factor.

Now, since the major change in the trend towards more white occurs during the period when the dam has not yet completed her growth (nearly complete at 15 months), Wright[1] concluded that the effect of age of dam on the piebald pattern "must thus be considered an immaturity rather than a senescence effect."

Polydactyly. The atavistic occurrence of a little toe on the hind foot of guinea pigs results in four hind digits instead of the normal three. The hereditary basis of this character was investigated by crossing an inbred strain (Strain D), breeding true to perfect development of the little toe, with three strains breeding true to the three-toed condition (Strains 2, 13, 32), as well as with another strain (35) which produced approximately 31 per cent four-toed offspring.[3] Depending on which strain was mated with D, the breeding results indicated strain differences varying from one to four Mendelian factors of comparable importance. It is of interest in this connection to note that crosses between D and 2 gave results in F_1, F_2, and backcross very similar to those expected if the three-toed condition were due to a single dominant Mendelian factor. Only when the supposed segregants were subjected to further backcross tests did it become evident that this simple explanation could not account for the inheritance of polydactyly.

TABLE 1

CORRELATION BETWEEN AGE OF MOTHER AND AMOUNT OF WHITE SPOTTING IN MALE AND FEMALE OFFSPRING

(From Wright, 1926)

Age of dam, months	Males		Females	
	No.	Per cent white	No.	Per cent white
3.0–	182	56.3	153	60.5
6.0–	195	59.5	187	67.6
9.0–	152	60.6	160	66.5
12.0–	150	61.3	124	70.6
15.0–	174	63.2	149	69.6
21.0–46.0	138	66.9	144	73.3
Total	991	61.1	917	67.8

A comparison of the results of mating three-toe × three-toe and four-toe × four-toe animals within given substrains (of Family 35), with both types of matings producing four-toed offspring in about the same frequency, indicated that, for animals of the same substrain, differences in toe number must be due almost entirely to nongenetic factors. Additional analysis indicated a marked tendency to concurrence of polydactyly in littermates. Correlated with this concurrence was, again, the age of the mother, increasing age correlated with decreasing frequency of polydactyly in the offspring. This relationship is shown in FIGURE 1.[2]

Conclusion. Variations in both piebald pattern and polydactyly in guinea pigs were shown by Wright to depend on the age of the mother. In both cases, moreover, the change in expression of the character was most marked when the mother had not yet reached maturity (approximately 15 months). Whether the effect of maternal age on these offspring characteristics is due to competition between young mother and offspring for some substance, or whether it

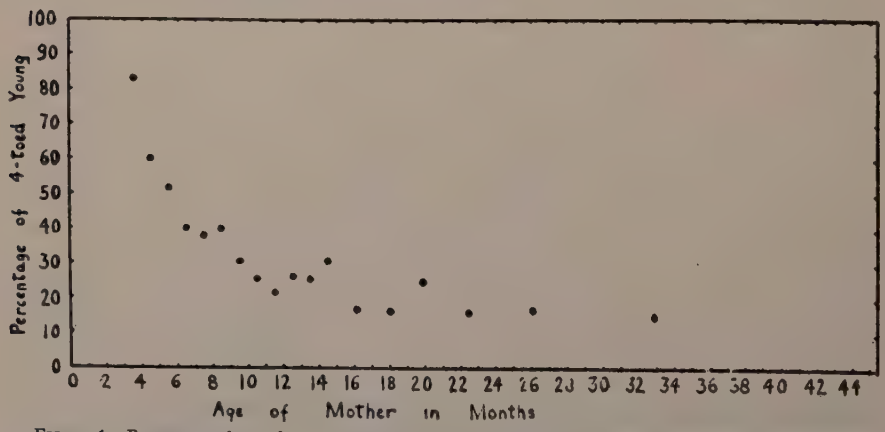

FIGURE 1. Percentage of 4-toed young according to age of mother. All points except first (3 months) based on more than 100 young.[2]

might be due to noncompetitive changes, perhaps hormonal, in maternal physiology during development to maturity, remains to be determined.

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MONGOLIAN IDIOCY (MONGOLISM) AND MATERNAL AGE

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The condition known as "mongolism," first described by Langdon Down[17] is an abnormality of human growth which presents one of the most baffling problems in medicine. The outstanding characteristics, severe mental retardation and physical dwarfing, are accompanied by widespread morphological disturbances. The nervous system,[1] the eyes,[20] the bones, the skin, the endocrine system, the vascular system, and the gut are all affected in a proportion of cases. The abnormalities are not always gross enough to be incompatible with fairly healthy life, but the expectation of length of life at birth is only about 12 years.

Mongolism has an incidence at birth in European populations of about 15 per ten thousand or one in 660.[5] Among Africans it is much rarer; by inference from the figures of Thompson,[33] it may be only one or two per ten thousand births. It occurs in Chinese and Indian populations with a frequency at present unknown but probably lower than the European rate. The most striking feature of the incidence is its relation to advancing maternal age. Early observers attributed this phenomenon to some kind of maternal exhaustion associated with excessive child-bearing. The birth order, however, by itself has no appreciable effect on the incidence.[24] There is often an unduly long interval between the birth of a mongol child and the preceding birth, but this is probably only a reflection of the same maternal age effect. The father's age is of no significance at all.[12, 23]

In a number of different embryonic and foetal abnormalities a relationship between incidence and increasing maternal age has been observed. In no example is the effect of maternal age as striking as in mongolism. For the sake of simplicity in demonstration, the proportion of children born after the mother has reached the age of 40 years may be taken as an index. Almost 40 per cent of mongol children come into this category. Other foetal or embryonic defects which have a similar maternal age influence are compared with mongolism in TABLES 1 and 2. Cystic degeneration of the chorion, malignant (chorion-epithelioma) or benign (vesicular mole), occurs chiefly at late ages and so also does central placenta praevia. In achondroplasia the effect is less marked. The tendency for cases of gross congenital malformations of the central nervous system to be born to mothers over 40 is still weaker. This tendency is thought, by Record and McKeown,[30] to be confined to hydrocephalus though anencephalus may have a slightly raised incidence also. In none of these examples has the phenomenon been so closely studied as in mongolism. The association of twinning with maternal age, however, first discovered by Duncan,[7] and afterwards shown to be almost confined to unlike-sexed pairs, was fully studied by Dahlberg.[6] The distribution differs from that found in the cases of foetal abnormalities because there is a maximal relative incidence for twins at the maternal age of about 37 years and thereafter a decline.

TABLE 1

COMPARATIVE MATERNAL AGE EFFECTS IN DIFFERENT ABNORMALITIES

Condition	Source of information	Total number of cases reported	Cases born after mother had reached age of 40 years	
			Number	Per cent
Mongolism	Brousseau[4]	584	224	38.4
	Penrose[29]	1,038	422	40.7
	Carter & MacCarthy[5]	100	35	35.0
Chorion-epithelioma	Brews[3]	24	8	33.3
Vesicular mole	Brews[3]	100	33	33.0
Central placenta praevia	Penrose[26]	35	8	22.8
	Kalmus[14]	32	4	12.5
Achondroplasia	Mørch[21]	99	15	15.2
	Krooth[15]	19	1	5.3
CNS malformation	Penrose[27]	144	17	11.8
Twins of unlike sex	Dahlberg[6]	12,818	804	6.3
Anencephaly	Scotland, 1939–1945: Registrar-General	1,671	93	5.6
Control population	England & Wales, 1939: Registrar-General	—	—	4.5
	Dahlberg[6]	—	—	4.5
	Scotland, 1939: Registrar-General	—	—	4.3
	Japan, 1948: W.H.O.	—	—	5.0
	U.S.A., 1936: W.H.O.	—	—	3.5

TABLE 2

SUMMARY OF MATERNAL AGE EFFECTS IN DIFFERENT ABNORMALITIES

Condition	Cases born after mother had reached age of 40 years (per cent)
Mongolism	39.5
Chorion-epithelioma	33.3
Vesicular mole	33.0
Central placenta previa	17.9
Achondroplasia	13.6
CNS malformations	11.8
Twins of unlike sex	6.3
Anencephaly	5.6
Control populations	3.5 to 5.0

The maternal age effect can be studied in detail by comparing the distribution of mongolism with that of the general population[12] or of an unaffected control group. These methods have also been widely used for studying the maternal age incidence of other foetal abnormalities.[22] They are open to the criticism that errors could be introduced if mothers of affected infants possessed some peculiarity not shared by the control mothers. For example, they might be disposed by constitution to produce children at relatively late ages. This

TABLE 3

MATERNAL AGE DISTRIBUTION OF MONGOLISM (ENGLAND AND WALES)

Maternal age in years	Absolute incidence*		Relative incidence	
	(a) Observed	(b) Expected	(a)/(b) Comparison with control	(c) Previously calculated from families
15–19	5	21.47	0.23	0.59
20–24	29	121.48	0.24	0.27
25–29	45	178.92	0.25	0.17
30–34	92	129.66	0.71	0.52
35–39	154	69.87	2.20	2.40
40–44	179	21.58	8.29	8.07
45–	41	2.02	20.80	15.07
All ages	545	545.00	1.00	1.00

* N.B. The incidence at birth in the general population is 0.15 per cent.
(a) 445 cases from Penrose[29] plus 100 cases from Carter & MacCarthy.[5]
(b) Population sample, England & Wales 1939, derived from Registrar-General's Report.
(c) Derived from 224 cases, Penrose.[24]

TABLE 4

MATERNAL AGE DISTRIBUTION OF MONGOLISM (ENGLAND AND WALES)

Maternal age	Relative percentage incidence at birth	
	(a)/(b)*	(c)†
15–19	0.03	0.09
20–24	0.04	0.04
25–29	0.04	0.03
30–34	0.11	0.08
35–39	0.33	0.36
40–44	1.24	1.21
45–	3.12	2.26
All ages	0.15	0.15

* Comparison with control.
† Sibship calculation.

objection is met if the control group is built up from the brothers and sisters of affected cases by suitable statistical procedures although, even then, there is a possibility of errors being caused by voluntary family limitation. On the whole, there is good agreement between the results obtained by the various methods with respect to mongolism. This is shown in TABLE 3 which refers to data collected in England. The actual incidence at birth for each maternal age group is shown in TABLE 4; the figures here are obtained by multiplying the corresponding ones in TABLE 3 by 0.15 per cent.

Detailed inspection of TABLE 2 shows that the risk of the birth of a mongol child for mothers below the age of 30 years is small, that is, less than one in two thousand. There may be a slightly increased chance at very young maternal ages. This point is difficult to decide because of the small numbers of control figures. After the maternal age of about 27 there is a rapid and continuous

rise in relative incidence, representing a risk for mothers which has a nearly threefold increase every five years. There is no indication of slackening even at late maternal ages. Several different estimates have been made of the maximal figure reached. Again, small numbers produce uncertainty, but, taking together the evidence provided from various sources and also from TABLE 3, an estimate of between 2.5 per cent and 3 per cent would seem not unreasonable. The relative flatness of the curve of distribution of absolute incidence (a), before the maternal age of 30, requires comment because it may indicate that there are two types of causation. Although the curve is not bimodal, it shows significant evidence of bitangentiality.[9] In a fairly large proportion of the cases, say one third, maternal age may not be an appreciable factor in the causation.

Instances of more than one case of mongolism in a group of close relatives occur more often than is generally believed, as was first shown in the survey made by van der Scheer[32] in Holland. It is difficult, nevertheless, to be sure that familial examples occur more frequently than would be expected on the hypothesis of chance coincidence. In my own survey[25] of the sibs of mentally defective patients I found that mongolism was about ten times as common among the sibs of mongol cases as among sibs of other types. I believe this figure to be too high for a general estimate owing to selection of material. More probably the incidence in sibs of affected subjects is not more than two or three times the population incidence and therefore very difficult to detect. However, there is evidence for genetical causation from other sources. Lelong *et al.*[18] reported an affected mother and son although, in Sawyer's[31] case, an affected mother had a normal daughter. The condition is so lethal that, up to the present time, these are the only two examples of fertility in mongolism accurately recorded in the whole medical literature. Twin data have been taken to indicate that mongolism has a genetical background because there are numerous instances on record of monozygotic twins both affected. There is no example known where only one of a monozygotic pair is affected though, in pairs judged to be dizygotic, a normal partner is usual.[13] Further evidence of genetical influences comes from observations on abortive forms or microsymptoms found among close relatives of affected cases.[34] This type of evidence, which, at present, is not very reliable, concerns the unduly frequent occurrence of traits, characteristic of mongolism but not necessarily abnormal, in parents and sibs of the patients. Such traits are fissured tongue, transverse palmar line, unusual position of the palmar axial triradius and abnormal segmentation of leucocytes. The indications of peculiarities in normal members of these families, when compared with the general population, can only be demonstrated, if at all, in large numbers.[28]

Maternal age shows some interesting fluctuations in familial cases. (FIGURE 1.) These peculiarities are summarized in TABLE 5. In families containing two affected sibs the mean maternal age for affected children is lower than that for the general run of cases of mongolism. The mean maternal age for affected sibs is two years less than that of a sample of unrelated cases. A similar and more significant reduction in mean maternal age occurs in cases

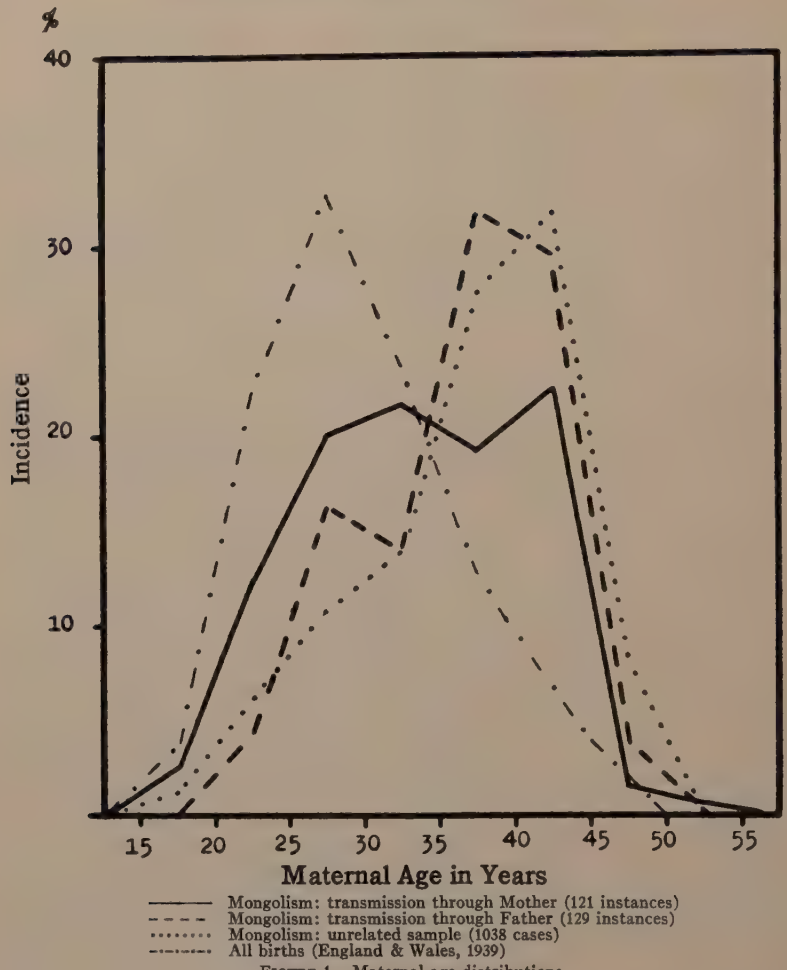

FIGURE 1. Maternal age distributions.

TABLE 5
MEAN MATERNAL AGES IN DIFFERENT GROUPS OF CASES

Sample	Number	Mean maternal age in years	Standard deviation
(i) General population (England & Wales)	—	28.6	6.0
(ii) Mongolism: sib also affected	70	34.3	7.4
(iii) Mongolism: maternal relative affected	121	33.0	7.3
(iv) Mongolism: paternal relative affected	129	35.8	6.3
(v) Mongolism: no known relative affected	1,038	36.6	7.1

t-values of comparisons of means: (i) with (ii), 6.4; (i) with (iii), 6.6; (i) with (iv), 13.0; (i) with (v), 33.3; (ii) with (iii), 0.6; (ii) with (iv), 1.4; (ii) with (v), 2.5; (iii) with (iv), 3.2; (iii) with (v), 5.2; (iv) with (v), 1.3

who have a maternal relative affected. These changes could be due to the inclusion of larger numbers of cases born at normal maternal ages than is usual in mongolism in groups (ii) and (iii); about two thirds could have a normal maternal age distribution. No reduction of mean maternal age is noticeable when a paternal relative is affected. Furthermore, in the data I have personally examined, no peculiarity could be detected in the means or distributions of the ages of the grandparents at the births of parents of cases.

Some observers have drawn attention to the excess of male cases found regularly in institutional populations and have asserted that affected males are altogether more common than females from birth onwards.[11] Concensus of opinion is against this view. The sexes are about equally likely to be affected and no significant difference between them in respect of maternal age can be demonstrated. Furthermore, pairs of affected sibs of like sex are no more frequent than those of unlike sex. Altogether there is no indication of any appreciable degree of sex limitation and no evidence for the supposition of sex linkage entering into the causation.

The susceptibility to mongolism may be partly genetically determined and, if so, then the underlying constitution, though rarely manifested, must be common. This is shown by the high incidence in the general population at late maternal ages where manifestation of the genetical influence may be nearly complete. It is also implied by the low familial concentration of either fully affected or abortive cases. It is most natural, however, to suppose that some infants are more susceptible by virtue of their inherited constitutions than others to an unfavorable maternal environment. Alteration by genes of the threshold of resistance may come about in various ways. When an inherited susceptibility is widespread, the distinction between dominant and recessive genes is difficult to make. Moreover, Grüneberg[8] has observed in mice that a genetical difference, whose manifestation is greatly influenced by environment, is likely to be polymeric. According to Hanhart,[10] the genetical background of mongolism is a single dominant gene. I have preferred the hypothesis of a single recessive gene as a first approximation even though it implies a gene frequency of at least one in five. There is, however, no evidence excluding the view that susceptibility is determined by a single common intermediate gene or by several common genes with alternative and additive effects. The mode of action of the maternal environment is entirely obscure. The mothers of cases of mongolism show no consistent disabilities and their general health is no worse than the average. If an endocrine disturbance is to be held responsible for all cases, it must be of a kind not yet known to clinical medicine.

Another plausible hypothesis is that the chief etiological factor is in the maternal rather than in the fetal constitution. There might be a recessive gene, which rendered the homozygous mother liable to have affected offspring in a manner analogous to that postulated by Bonnevie and Sverdrup[2] to account for human dizygotic twinning. The apparent significance of paternal inheritance in many families would be unexplained unless this maternal hypothesis is combined with one which assumes that the fetal genotype is also

significant. Mongolism would then be the result of a complex interaction between mother and fetus depending upon both their genotypes as well as upon other factors. The reduction of mean maternal age, in cases where the mother apparently transmits the hereditary influence, suggests that similarity in constitution between mother and offspring is a predisposing cause. This observation agrees with what has been found by blood typing. There is no tendency, for example, for *rhesus* incompatibility to occur in mongolism. For *rhesus* and some other antigens, the affected child tends to be slightly more compatible with the mother than would be expected on the basis of random sampling.[16] It is clear that the interaction responsible for mongolism cannot be due to the incompatibility of any known red cell antigens.

The possibility that mongolism is due to fresh mutation each time it occurs can probably be excluded on the grounds of this being too common an event. Nevertheless, some rather more frequent process simulating fresh mutation cannot be altogether excluded as an explanation; for example, a rare type of crossing over of the kind described by Lewis[19] between two closely linked genes might be the sole cause. Maternal age could be reasonably assumed to influence the rate of crossing over although it is difficult to see how it could alter mutation rate in any marked degree. Another explanation worthy of mention is that mongolism is an effect due to unbalanced chromosomes caused by translocation. This would explain collateral transmission and the low familial incidence but not the maternal age effect. Again, perhaps the cause might be a cytoplasmic agent which developed slowly in the maternal germ cells. This could explain the maternal age effect but would imply that the father could hardly ever be a transmitter.

Finally, it is held by many investigators that there is no genetical background at all determining mongolism and that purely accidental environmental circumstances are to be blamed. A fluctuating endocrine disturbance of gradual onset in the mother could explain the maternal age effect in general. Exceptional shocks or illnesses would have to be postulated to account for the origin of cases at young maternal ages. The absence of any special type of maternal ill health, associated with pregnancies terminating in the birth of affected infants, seems to indicate that the maternal influence responsible must be related to physiological rather than to pathological changes.

It has almost always been assumed, without question, that the effect of increasing age in mongolism is to produce more abnormality. It is conceivable that the reverse is true, namely, that, in young mothers, pregnancies do not result in affected children because the disease is so severe that it does not allow the fetus to develop beyond the very earliest stages. Only in later maternal life does the disease become mild enough to allow mongolism to appear in a surviving infant. The main objection to this view is that the mothers of mongols do not have appreciably more miscarriages than control mothers. Also, I have attempted without success to demonstrate a significant relationship between maternal age and severity of malformation or viability among affected children. On the other hand, this reverse hypothesis would accord well with the maternal age effects described in animal genetics[35] where younger mothers

have the more abnormal offspring. Little seems to be known about the outcome of very late pregnancies in wild populations of animals as opposed to laboratory stocks. If the distributions shown in TABLE 3 were found to have a parallel in some animal other than man, the hypothesis of improvement of human offspring with advancing maternal age, rather than worsening, would seem even less plausible than it does at present. The difficulty of resolving even this outstanding question with certainty serves to emphasize once more the extremely complex nature of the problem of the etiology of mongolism.

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THE BIRTH OF CONGENITALLY MALFORMED CHILDREN IN RELATION TO MATERNAL AGE

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Introduction

The living organism deteriorates somatically as it grows older, and aging ultimately terminates its reproductive capacity. It ought not to be surprising, therefore, if aging also should have a deteriorating effect upon the germ cells, just short of the time that reproduction becomes impossible.

That aging may have such an effect is suggested by the high frequency with which mongoloid children are born late in parental reproductive life. If it can be assumed that aging of the germ cells plays a role in the etiology of mongolism, such a hypothesis would suggest that it also might influence the frequency with which other congenital defects might occur. The present report deals with this premise.

Materials and Methods

A study was made of the outcome of every conception of a series of mothers each of whom had a congenitally malformed child.[1] The mothers were located through the medium of the death certificates of their defective offspring.

The diagnosis of a defect was considered to have been satisfactory, either if the malformation involved the body surface or, if solely internal, it had been disclosed by operation or autopsy.

Results

The ages of 570 mothers at the births of 607 of their congenitally malformed children, and of 1,584 of their normally developed offspring, were determined. The relation between maternal age and the development of the offspring is discussed under the following headings:

(1) Maternal age at the birth of the first normal and at the birth of the first defective child.

(2) Earlier *versus* later marriage and the intervals before the birth of the first normal and first defective child.

(3) The ratio of defective to normal offspring at various maternal ages.

1. Maternal age at the birth of the first normal and at the birth of the first defective child. There were 466 families each of which contained one malformed child and at least one normally developed one. The average age of the mothers at the time of marriage was 21.1 years. At the birth of the first normal child the mothers averaged 23 years of age. The first defective child, however, was not born until the average maternal age was 28.4 years. In other words, the first defective infant appeared approximately five years after the birth of the first normal child.

2. Earlier versus *later marriage and the intervals before the births of the first*

TABLE 1

EARLIER VERSUS LATER MARRIAGE AND THE INTERVALS BEFORE THE BIRTH OF FIRST NORMAL AND FIRST DEFECTIVE CHILD

Mothers number	Average age at marriage (years)	Average number of pregnancies	Average interval from marriage to birth of first	
			Normal child (months)	Defective child (months)
45	16.8	3.08	15.3	87.2
45	26.8	2.09	21.0	59.0

The average intervals between marriage and the birth of the first normal and of the first defective child, respectively, for two groups of mothers whose average age at marriage differed by ten years. Note in the last column that the average interval between marriage and the birth of the first defective child was longer in the case of the mothers who married earlier than for the ones who married ten years later.

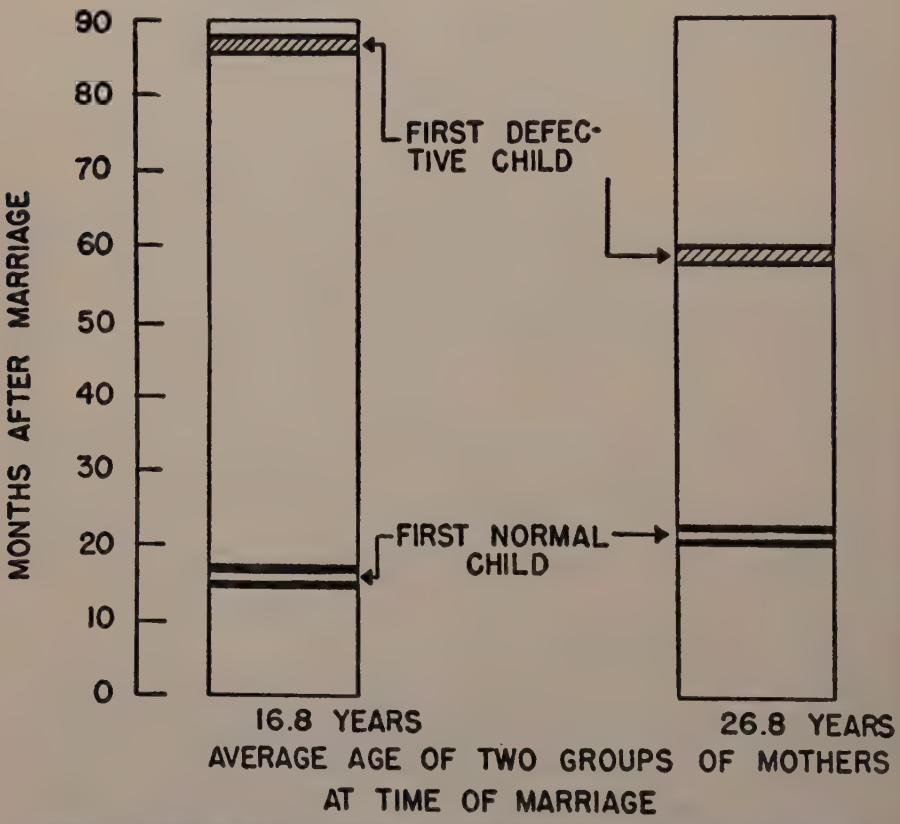

FIGURE 1. Graphic presentation of data shown in TABLE 1. The vertical scale indicates number of months following marriage. The left bar represents 45 mothers whose average age at marriage was 16.8 years; the right bar represents 45 mothers whose average age at marriage was 26.8 years. The marriages of both groups took place at point zero on the vertical scale. The unmarked horizontal bars mark the average number of months after marriage at which the birth of the first normal children took place. The cross-hatched bars mark the average number of months after marriage at which the first defective children were born. Note that the intervals in months between marriage and the births of the first defective children was greater in the case of the women who married earlier. Also note that the intervals between the births of the normal and the defective children were longer for the women who married earlier.

TABLE 2

CHILD DEVELOPMENT BY MATERNAL AGE

Ages of mothers (years)	Defective		Normal		Col. 3 divided by col. 5
	Number	Per cent	Number	Per cent	
(1)	(2)	(3)	(4)	(5)	(6)
10 to 14	0	—	1	—	—
15 to 19	43	7.1	130	8.2	0.866
20 to 24	163	26.8	505	31.9	0.840
25 to 29	176	29.0	516	32.6	0.890
30 to 34	111	18.3	277	17.5	1.045
35 to 39	86	14.2	123	7.8	1.820
40 to 44	24	4.0	28	1.8	2.241
45 to 49	4	0.6	4	0.2	3.000
Total	607	100.0	1,584	100.0	—

The distribution of defective children, and of their normally developed siblings, by maternal age at the time that the children were born. Note in column 6 that the ratio of defective children to their normally developed siblings increased directly with maternal age.

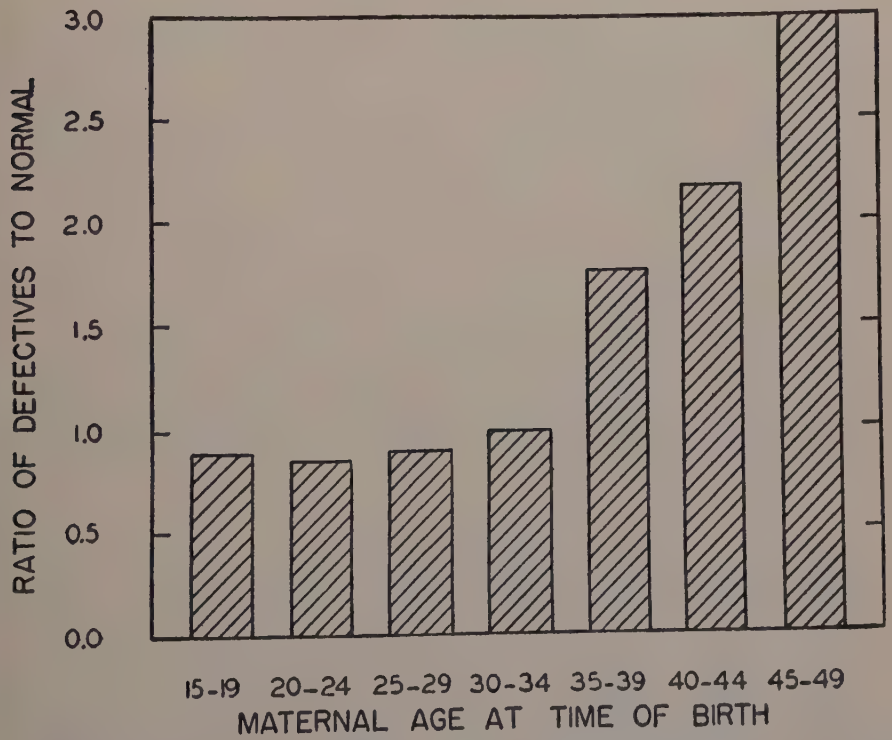

FIGURE 2. Showing ratio of defective children to normally developed sibiings according to maternal age at time of birth, based upon data taken from TABLE 2, column 6. The base line records the ages of mothers at the births of all children. The vertical line denotes the ratio of the defective children to their normal siblings. Note that (1) the proportion of defective to normal offspring was relatively constant for the mothers who were under 30 years of age, (2) the proportion of defective children increased progressively after the mothers reached 30 years of age, and (3) after the mothers were 45 years of age the ratio of defective to normal children was three or more times as great as it was before the mothers were 30 years of age.

normal and the first defective child. The interval between marriage and the birth of the first normal child, and the interval between marriage and the birth of the first defective child were compared for two groups of 45 women each, whose average ages at marriage were separated by an interval of ten years as shown in TABLE 1 and FIGURE 1. One group of mothers was married at an average age of 17 years, the other group at 27 years of age.

The mothers who were married at the earlier age had an average of 3.08 pregnancies, whereas whose who were married later had an average of only 2.09 pregnancies. The first group (married earlier) had their first defective children, on the average, 87 months after marriage, whereas the second group (married later) had their first defective children only 59 months after marriage.

In the case of the mothers who married earlier, the average interval between the birth of the first normal child and that of the first defective child was 72 months, whereas for the mothers who married later, it was only 38 months. In other words, after the births of their first normal children, the women who married earlier waited nearly twice as long before giving birth to their malformed children as did the mothers who married later.

3. The ratio of defective to normal offspring at various maternal ages. As was mentioned above, the ages of 570 mothers at the births of 2,191 of their normal and defective offspring were known. Six hundred and seven of their children were malformed, and 1,584 were normally developed at birth. The defective and normal offspring are distributed according to maternal age in TABLE 2. The ratio of the defective children to their normally developed siblings is shown in TABLE 2, column 6, and in FIGURE 2, according to the maternal age at the time of birth.

The data in TABLE 2 indicate that the proportion of defective to normally developed children remained more or less constant for births occurring between maternal ages of 15 and 29 years. Beginning at the age of 30, or even a little earlier, however, the proportion of defective children increased.

Each succeeding five-year period reveals a progressive increase in the proportion of defective children. When birth took place between 45 to 49 years of maternal age, the proportion of defective to normal children was approximately three times as great as that observed when the mothers were under 30 years of age.

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THE CUMULATIVE EFFECT OF LITTER SERIATION ON FIBROSARCOMA SUSCEPTIBILITY IN MICE*

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It has been shown that some principle in the mother's body, as measured by litter seriation, has an effect upon susceptibility to and characteristics of chemically induced fibrosarcomas in mice.[1-8] This principle increases with the progressive aging of the parents and is selectively handed down to their offspring by some nongenic means. The discovery of this aging principle was made, as a collateral observation, when the influences of the carcinogen, methylcholanthrene, upon germinal mutations and cancer susceptibility were under investigation. The gene *b* (recessive brown) had mutated to *B* (dominant black) eight times following the injection of methylcholanthrene into a series of breeding mice. This mutation occurrence was so high the suggestion became apparent that perhaps the mutation rate was increasing with a greater exposure to the carcinogen. It became desirable, therefore, to keep mice, injected with methylcholanthrene, breeding as long as possible and to examine their offspring closely. It was found that the chemically induced mutation rate at specific loci (*b–B*) did not increase as the carcinogen-treated parents grew older, but that the appearance of fibrosarcomas at the site of the injection of methylcholanthrene did increase in frequency. Mutations at loci other than at "*b*" arise, however, at the rate of about 1 to 700 mice following the subcutaneous injection of methylcholanthrene, whereas the spontaneous mutation rate is 1 to 24,000, but these chemically induced mutations do not increase with the advancing age of the parents.[9, 10]

The present paper will deal with two problems: (1) The presentation of new data which demonstrate that the effect of litter seriation (mother's age?) upon chemically induced fibrosarcomas in mice is cumulative over a period of generations. The characteristic of latent period (time between the injection of methylcholanthrene and the appearance of fibrosarcoma) shall be the only characteristic of malignancy considered. (2) The discussion of the present data in terms of applying them, if possible, to the analysis of cancer frequency in the human population.

Materials and Methods

Only one inbred strain of mice has been used in this investigation. This is the 2-Prunt whose origin has been discussed previously.[6] Since the preceding report, ten generations of inbreeding have been added to this descent.[8] Thus, the mice have reached the fortieth generation of brother to sister matings. Two sublines of the 2-Prunt have been separated off from the original inbreds (see FIGURE 1). One of these (the advanced litter descent) has descended from parents of the 2-Prunt strain which belonged to seventh, eighth or ninth litters.

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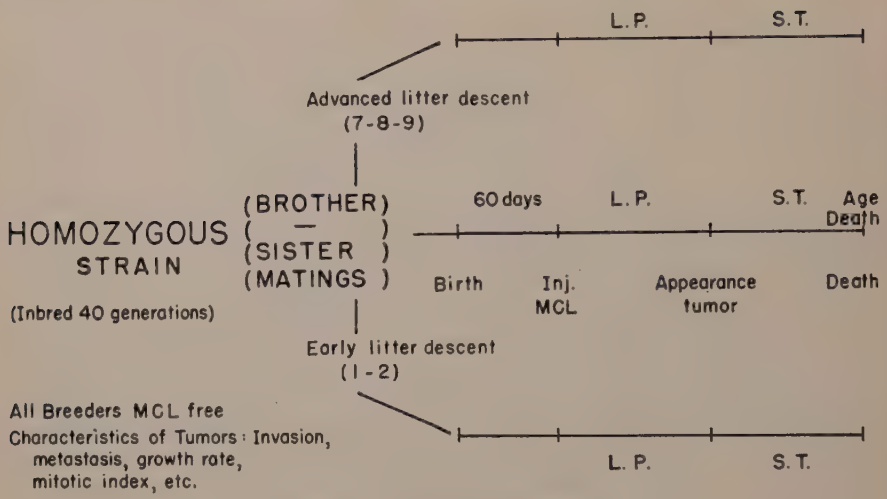

FIGURE 1. The plan of the experiment.

The second subline has descended from parents which belonged to first or second litters only. The advanced litter descent was terminated after three generations, whereas the early litter descent has been continued for more than ten generations of early litter selection. The original 2-Prunt descent is also being continued.

At sixty days of age, all mice (except those that were used for breeders) were injected with one milligram of methylcholanthrene dissolved in 0.1 cc. of sesame oil. All mice were kept until death either from the growth of the fibrosarcoma arising at the site of the injection of the carcinogen or from some other cause, such as tumors arising at other sites, intercurrent disease, emaciation, *etc.* The present analysis is based exclusively upon locally appearing fibrosarcomas, verified by histological examination.

In an earlier report on litter seriation phenomena in fibrosarcoma susceptibility,[6] 341 female mice and 265 males had developed fibrosarcomas as the result of the injection of methylcholanthrene and had died. To this series 668 new mice, belonging to various litters have been added. Of these 376 were females and 292 males, thus making a total, in both series, of 717 female mice developing fibrosarcomas and 557 males developing the same type of tumor. The advanced litter descent consisted of 212 females and 134 males developing fibrosarcomas. In the early litter descent, 186 female mice have developed fibrosarcomas. (At the same time 55 males belonging to several litters of the early litter descent have developed fibrosarcomas, but this number is not sufficient for the present analysis of the latent period and they are not included in this report.) Thus a total of 1806 mice is included in this paper, of which 1115 were females and 691 males. These mice are divided between the first to the eleventh litters. The data on advanced litters are still small. It is demonstrated in the present paper that the three sublines of the original inbred strain (after forty generations of brother-to-sister matings) are beginning

TABLE 1

	♀	♂
Old series	341	265
New series	376	292
Total 2-Prunt	717	557
Advanced litter descent	212	134
Early litter descent	186	0
Total	1115	691

The data on the number of mice used in the present investigation. The original 2-Prunt descent and the two derived sublines are given separately.

TABLE 2

	Median latent period	Sex difference
1. ♀ Total 2-Prunt	118.0 ± 1.16	
♂ " "	176.0 ± 2.89	58.0 ± 3.11 or 18.6 × P.E.
2. ♀ Adv. litter des.	126.0 ± 2.13	
♂ " " "	164.0 ± 3.79	38.0 ± 4.34 or 8.7 × P.E.
3. ♀ Early litter des.	98.0 ± 1.56	
Differences		20.0 ± 5.34 or 3.74 × P.E.
♀♀ 1–3	20.0 ± 1.94 or 10.3 × P.E.	
2–3	28.0 ± 2.64 or 10.6 × P.E.	
1–2	8.0 ± 2.42 or 3.3 × P.E.	

The analysis of the data by the formula

$$\text{P.E. Median} = \frac{\text{Average deviation of quartiles}}{\sqrt{n}}$$

where n is the number of determinations. The data on all litters are combined in this analysis.

to diverge. For this reason, no more of the original first series of the 2-Prunt are being added.

Results

The data on the latent periods, expressed as medians, are presented in TABLES 1 and 2. In these tables, the data on all litters are tabulated together. These same data are also presented graphically in FIGURE 2, where the data on the successive litters are kept distinct. Previous data have shown that in the original 2-Prunt descent there was a significant sex differential in relation to the median latent period (time between the injection of the methylcholanthrene and the appearance of the fibrosarcoma) which was maximal in early litters but which gradually diminished in mice of successive litters due mostly to an increased susceptibility to fibrosarcomas in successive litters of males (slope of latent period line was 5.5). In advanced litters of the original 2-Prunt descent there was no sex differential in relation to this cancer susceptibility characteristic. The over-all sex differential in the original 2-Prunt descent is, at present, 58.0 ± 3.11 days which is 18.6 × P.E. These observations on the reduction of the sex differential in relation to fibrosarcoma susceptibility has now been verified with new data. Mice of the advanced litter descent (from parents of 7, 8 and 9 litters where the sex differential had been practically diminished) show an over-all sex differential for chemically induced fibrosarcomas of 38.0

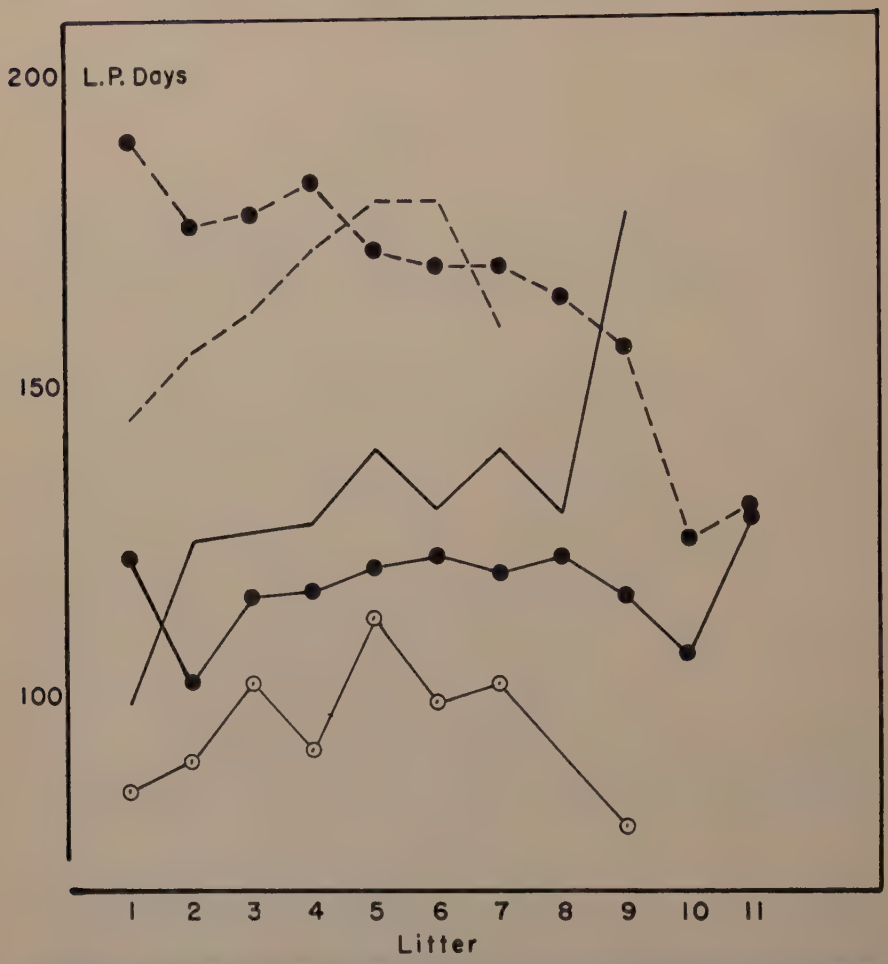

FIGURE 2. This presents graphically the same data given in TABLES 1 and 2, except that the data on successive litters are separated. The average latent periods are given on the vertical line and successive litters (or litter seriation) along the base line. Data on the sexes are given separately (females on solid lines and males on dash lines. The original 2-Prunt strain on females solid and solid circle lines, males dash and solid circle lines; females of advanced litter descent on solid line and males on dash line. Data for female mice of the early litter descent are given on the solid and open circle line.

$\pm$ 4.34 days or 8.7 $\times$ P.E. The sex differential is still significant, but it has been diminished significantly from the value obtained for their own parents by 20.0 $\pm$ 5.34 days which is 3.74 $\times$ P.E. The reduction in the sex differential, however, is based, to a great extent, on the fact that the fibrosarcomas in the advanced litter descent come significantly later in daughters (126.0 $\pm$ 2.13 days) than they did in their maternal relatives (118.0 $\pm$ 1.16). The tumors in the sons come somewhat earlier than they did in the paternal relatives, 164.0 $\pm$ 3.79 compared to 176.0 $\pm$ 2.89. However this last difference in the two male groups is not significant. The daughters of the advanced litter descent

have deviated significantly from the maternal line by 8.0 ± 2.42 days, which is 3.3 × P.E.

In the early litter descent, the median latent period for fibrosarcomas in the females was 98.0 ± 1.56 days. This is a deviation from the maternal descent of 20.0 ± 1.94 days which is 10.3 × P.E. The females of the two selected descents (the advanced litter descent and the early litter descent) have deviated from each other by 28.0 ± 2.64 days or 10.6 × P.E.

Thus divergence in a biological characteristic (susceptibility to a chemically induced fibrosarcoma as measured by the latent period) has been obtained starting with an inbred or homozygous stock of mice (after 30 generations of brother to sister matings) and continued exclusively by brother to sister matings. Selection which has brought about this divergence is based upon the exclusive fact whether the parents, grandparents, and more remote generations have belonged to first and second litters in one line or to seventh, eighth, and ninth litters in the other line.

General Discussion

The discussion of the present data may be appropriate to several biological fields. These may be classified, as follows:

(1) Cancer research;
(2) General biology,
  a. Gerontology,
  b. Genetics,
  c. Evolution;
(3) Re-evaluation of origin of inbred mice;
(4) Application to the human cancer problem.

Before discussing the bearing of these new data upon the above biological fields, it would be desirable to outline, briefly, the facts obtained. These facts are as follows: There is apparently a principle in parents which changes progressively during the aging process and is handed down to the offspring. This transmission is apparently by some nongenic means and susceptibility to and characteristics of malignancy (chemically induced fibrosarcoma in mice) are significantly affected. The principle, whatever its nature eventually proves to be, appears to have a small effect on susceptibility to fibrosarcoma in mice of successive litters, but, being cumulative over a period of several generations, its effect upon fibrosarcoma becomes highly significant. The application of this principle to other biological phenomenon would depend upon several facts which are still not available. Among these data not known at present are: (1) whether the phenomenon of litter seriation is reversible as Lansing determined in longevity of rotifers and (2) whether the characteristics of a species have been permanently affected. That is, if these deviations are only of a temporary nature and, by reversing the method of selection used, the original susceptibility to fibrosarcoma could be obtained, then one biological process may be involved. On the other hand, if a divergence of a biological characteristic is permanent, then some other biological interpretation would be indicated. At present, it is unwise to reverse selection of parents based upon the

litter to which they belong. This attempt of reversed selection should not be done until static equilibrium (a constant median value of the latent period) in the two selected sublines can be obtained. This constancy of fibrosarcoma susceptibility has probably not yet been obtained.

A situation somewhat similar to the present one in the shift of fibrosarcoma susceptibility was reported by Pearson in "Size Inheritance in Man." His well-known "law" that "the offspring of extremes tended toward mediocrity" could very well be applied to the present problem. There is one difference, however, (which may be fundamental) between size inheritance in man and fibrosarcoma susceptibility in mice. This is the progressive change with the age of the parents, which applies only to fibrosarcoma susceptibility in mice. In either analysis, the effect of biological shift seems to be to keep the species constant. Pearson's data on size inheritance have been adequately interpreted in terms of multiple factors. Whether the aging process of parents in fibrosarcoma susceptibility in the offspring can be explained in the same terms of multiple factors appears, at present, to be extremely difficult but not impossible. So far as is known, a genic mechanism of transmission from parent to offspring does not change during the aging process, but cannot be ruled out on *a priori* grounds. The fundamental biological nature of this changing principle in parents and its transmission to offspring which determines fibrosarcoma, however, is not yet determined, and its interpretation is purely conjectural.

Tumor susceptibility in experimental animals appears to be complex. There are, in addition to this "nongenic principle" which changes with the age of the parents, specific genes, particularly the gene on the *s*-chromosome which influences fibrosarcoma susceptibility in mice.[11]

In this monograph, it is proposed to restrict further discussion to the possibility of applying to man the principles of the aging process in parents to susceptibility to neoplasia in the offspring.

Two points must be kept clearly in mind. (1) It is a truism that not all tumors are alike. The evidence is convincing that, in man, we may be dealing with a multiplicity of diseases. (2) This diversity of neoplasia also applies to experimental cancer.

So far, the effect of the aging process upon experimental cancer susceptibility has only been demonstrated in three neoplasia and all three of these appear to be biologically different. These are as follows: (a) MacDowell and Law have demonstrated a decreasing incidence of leukemia in mice with advancing age of the parents; (b) Bittner and others have shown that adenocarcinoma of the mammary gland in mice increases with advancing age of the mother but they interpret their findings in terms of an increased potency of the milk factor; and (3) The present work considers chemically induced fibrosarcoma in mice which may either increase or decrease in different strains of mice with parental age and in which there appear to be no milk factor.

It is commonly maintained that part of the increase of cancer in man is due to the increased expectancy of life. This correlation may be a valid one but leaves much unaccounted for. As in all biological characteristics, particularly the so-called quantitative ones such as growth, size, pigmentation, *etc.*, there are many influences, some intrinsic or genetic and some environmental, that

shorten or lengthen the span of life. In order to ascertain which one of these, if any, influences cancer susceptibility, it becomes desirable to analyze all forces on longevity. Most of the increase in life expectancy during the twentieth century is due to the control of the diseases of childhood; part is due to improvements in medical practice and to sanitation. The antibiotics may possibly lengthen life but their effect on the average expectancy of life cannot, at present, be determined. It is probably true that a man who lived sixty years toward the beginning of the twentieth century would not live much beyond that age now. So that the nature of man has probably not changed while this increase of cancer and cancer deaths has been going on. Beeton, Yule, and Pearson,[13] however, have maintained that there has been a selective effect on longevity during the seventeenth to nineteenth centuries by the fact that a large proportion of the population is made up of large families produced by parents who had lived to advanced ages.

There is a rich literature on the forces at play on longevity in man, but the information desirable on the point at issue of the aging process on cancer susceptibility and the biological characteristics of the ordinal sequence of the offspring is not available. It is the present intention to deal with a few references which may be of interest in the problem of cancer susceptibility. Jalavisto[12] has shown that the life expectancy of both male and female children is shortened by the increased age of the mother at the time the children were born. This effect on longevity does not apply to the age of the father. Thus a maternal effect on life expectancy is indicated. There is a possibility that the phenomenon of litter seriation on fibrosarcoma susceptibility may be due to maternal or "cytoplasmic" inheritance.

Beeton, Yule, and Pearson[13] have shown that the number of children produced in the Connecticut Whitney family is correlated with the age of both the mother and the father. They conclude, "in the course of our investigations we have seen that the relationship between fertility and duration of life does not cease with the fecund period. We thus reach the important result that characters which build up a constitution fittest to survive are also characters which encourage its fertility. This result is of great value from the standpoint of the differentiation of type where it is absolutely necessary that the fittest to survive should also be the most fertile. On the other hand, we note that duration of life is a character capable of modification by reproductive selection, and we suggest that a considerable part of the increased expectation of life observed in recent years may be due to this cause. In the case of the American statistics we see at once how it can replace a remarkably short-lived stock by a longer-lived stock, the bulk of the offspring coming from the longer-lived members."[13]

The data of Beeton, *et al.* were based upon family records which were obtained before the beginning of the twentieth century. The great increased expectancy of life, however, has occurred after that time, during which period the average size of the native white family is gradually getting smaller.[16, 17] This reduction in family size may have a very significant bearing on an increased cancer incidence—providing of course that there be any difference in cancer susceptibility between the successive children (ordinal sequence) of the

same parents. The information on this point is not available but if there be any similarity of cancer susceptibility in man to chemically induced fibrosarcoma susceptibility in mice, this is what we should be looking for (litter seriation in mice may be compared to ordinal sequence of the children in man). The present data on fibrosarcoma susceptibility in the original 2-Prunt strain indicate a longer latent period for the appearance of the tumor for mice of the sixth litters compared to mice of the second litters. (124.0 ± 3.82 days for female mice of the 6th litters compared to 103.0 ± 3.64 days for female mice of the 2nd litters.) This difference is 21.0 ± 5.27 days or 4.00 × P.E. Now if these later litters were never obtained, it would appear that fibrosarcoma susceptibility had increased. It may be that cancer in man appears to be increasing, not exclusively by an increased expectancy of life, but by the fact that the size of the family is getting smaller.[16, 17] The decrease in the median latent period from 118.0 ± 1.16 days in the parental 2-Prunt inbred strain has been reduced to 98.0 ± 1.56 days merely by reducing the size of the family from 8–10 litters to 1–2 and continuing this early descent for several generations. Is not this reduction in potential fertility occurring in man now?

Ploetz[14] has shown that infant mortality (death before five years of age) decreases with advancing age of the parents. Johnson has recently shown that deaths from maternal causes have been reduced in recent years. These and many other factors must of necessity influence the average expectancy of life (if only for a minor effect). Some of these influences may very well be responsible for some increase of cancer in man.

Large families are still being produced, and it is the investigation of these that may provide the data upon which the analysis of cancer susceptibility and cancer frequency must be based. Lotke and Spiegelman[15] have shown that the age of the mother at the time of the birth of her children has changed very little between 1920–24 and 1934–38. This conclusion is based upon families of eleven and more children. The time element here is not sufficient to determine real trends. Certainly the present study of experimental cancer indicates that perhaps the age of the mother at the time the child was born may have a significant influence upon cancer susceptibility. Lotke has indicated a conclusion from his work which should not be lost sight of. He concludes, "Any intensive study of the movement of population must necessarily include, as one essential, an examination of the separate components of the total reproductive performance along such lines as here set forth."

Plan for Analysis of the Incidence of Cancer in Man

There appear to be at least two practical means of attempting to prove whether the phenomena of litter seriation (or size of family and parental age) has any influence on the increased incidence of cancer in the human population. Several studies in man for the resolution of the complex longevity or expectancy of life are suggestive as the few above would indicate. As to the critical points at issue, (1) maternal (or paternal) age, (2) the size of family and (3) the biological potentialities in the ordinal sequence of the children, the literature fails completely. It has been indicated that the information we

desire to obtain is not available so that it becomes necessary to set up a program by which this information may be forthcoming.

These two programs are as follows: (1) The first program of investigating the possible effect of parental age upon cancer susceptibility in man has already been initiated by Doctors George Pack and Theodore Miller of New York City. By providing a list of questions on parental age, ordinal sequence of family, *etc.*, to cancer patients and to a group of controls, it may be possible to obtain a preliminary, if not a final, answer. Much should come from this attempt. The greatest weakness in the method appears to be in the possibility of obtaining data on patients belonging to small families whereas the important analysis should be based upon the study of large families so that the potentialities of children according to ordinal sequence of the family could be investigated. It may very well be that the final study should be based upon a few well-chosen types of tumors rather than upon all types. This program may indicate two trends that should be followed up by a second program of the study of the large family. The first program should indicate (1) any trends in specific types of cancer susceptibility similar to differences in experimental cancer that should be followed up; (2) the best types of human tumors that should be investigated; and (3) any instances where cancer has occurred in a member of a large family.

(2) The second program should be based upon the complete analysis of the large family, not only the biological potentialities of the ordinal sequence in relation to cancer but to other characteristics as well. Perhaps Doctors Pack and Miller have already reached the same conclusion.

Summary

The present study deals with the appearance of fibrosarcomas in mice following the subcutaneous injection of methylcholanthrene at sixty days of life. For this purpose, 1806 mice have been used, of which 1115 were females and 691 males. The mice belonged to one original inbred strain (30 generations) and continued exlusively by brother to sister matings. Three sublines have been continued (1) the original inbred and (2) and (3) two selected descents based upon whether the parents belonged to first or second litters (early litter descent) or the seventh, eighth, or ninth litters (advanced litter descent). Divergence in the appearance of fibrosarcomas has been obtained in the two selected descents which amount to 28.0 ± 2.64 days. This difference is $10.6 \times$ P.E. The evidence thus demonstrates that the principle, as measured by litter seriation (ordinal sequence of the family), which affects chemically induced fibrosarcomas in the offspring is cumulative.

The relation of the problem to the study of cancer frequency and increase in man is discussed and a new program of a cancer survey for the human population is proposed.

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INFLUENCE ON THE OFFSPRING OF ALTERED PHYSIOLOGIC STATES DURING PREGNANCY IN THE RAT*

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It is now well established that the course of embryonic development may occasionally be modified by environmental influences. The environment of the mammalian embryo can be thought of as consisting of two layers. First, there is the maternal body which provides remarkably constant physical and chemical surroundings for the embryo. Beyond the maternal body is what may be called the extramaternal part of the environment. This is not constant, but the fact is of little consequence because the maternal body is usually able to insulate the embryo against ordinary changes in the extramaternal environment. Only such uncommon influences as penetrating radiations are known to pass readily through the maternal barrier and directly affect the embryo. This is not to say that other influences arising outside the mother do not affect the embryo, but such outside agents must be mediated by the maternal organism, and the probability exists that many of them act indirectly by inducing some modification in the physiologic state of the mother. Thus, virtually all environmental change to which the embryo is likely to be subjected either arises within the maternal body or is mediated by it.

Regardless of whether internal or external in origin, changed physiologic states within the pregnant mother must have a place in any consideration of factors which may affect the offspring. Recent experiments and observations by several investigators have revealed that a number of agents are capable of acting on or through the pregnant mother to cause maldevelopment of her young. The varieties of these agents known at the present time as well as the diverse organs and systems of the embryo upon which they act are presented in TABLE 1. Although an impressive array, the fact remains that in no instance is the manner of action upon the embryo known. In the case of vitamin A[13] and pantothenic acid[1] deficiencies, the observation that the fetuses exhibited some of the symptoms of postnatal deficiency has been cited as evidence of direct action on the fetus. In these as well as in the other situations listed, however, the mothers experienced more or less profound physiologic alterations. The alterations were usually numerous and interrelated and it was not possible to relate any one or combination of them to the effect observed in the young. Indeed, more than one recent writer, after reviewing these observations, has suggested that any physiologic stress placed upon the pregnant mother might result in maldevelopment of her young if the stress were sufficiently intense.

In view of the need for further information on the subject, a series of experiments has been undertaken to study the types and degrees of maternal physiologic alterations capable of adversely influencing the development of the offspring. In general, a standardized procedure has been employed which calls for subjecting pregnant rats to a particular type of physiologic stress at a

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TABLE 1

AGENTS WHICH ACT ON OR THROUGH THE PREGNANT MOTHER TO CAUSE MALDEVELOPMENT OF THE OFFSPRING IN MAMMALS

Agent	Species	Organs or systems affected	Reference
Dietary deficiencies:			
Vitamin A	Pig	Eye, palate, kidney	Hale[5]
	Rat	Eye, lungs, cardiovascular, diaphragm, genitourinary	Warkany & Schraffenberger,[11] Wilson & Barch[12]
Riboflavin	Rat	Skeleton	Warkany & Nelson[10]
Pantothenic acid	Rat	Eye, brain, appendages	Boisselot[1]
Vitamin B_{12} (Folic a.?)	Rat	Eye, brain	O'Dell, Whitley, & Hogan[9]
Pteroylglutamic acid	Rat	Lens, palate, skeleton, (cardiovascular & urogenital)	Nelson, Asling, & Evans[8]
Nitrogen mustard	Rat	Palate, skeleton, appendages	Haskin[6]
Trypan blue	Rat	Eye, brain, spinal cord, palate, appendages	Gillman, Gilbert, & Gillman[3]
Hypoxia	Mouse	Skeleton, palate, heart, brain, lids	Ingalls, Curley, & Prindle[7]
Cortisone	Mouse	Palate & "variety" of others	Fraser & Fainstat[2]
Rubella infection	Man	Eye, internal ear, heart, brain	Grigg[4]

known period in gestation. Pregnancy was dated from the morning on which sperms were found in the vaginal smear of females mated on the preceding night. The method used to induce the stress was applied between the 7th and the 15th day of gestation in order to coincide with the period of most active differentiation and organogenesis in the rat embryo.

One form of physiologic stress thus far tested has been severe hemorrhagic anemia, which was produced by allowing free bleeding from the freshly severed tail on three successive days, beginning on days 7, 9, 11, or 13 of gestation. On the first day of bleeding, laparotomy was performed to verify pregnancy and to count the embryos. Hematocrit readings were made on each day of bleeding and on the day after the last bleeding. During the three bleedings total quantities of blood equivalent to 6 cc or more per 100 gm of body weight were removed. This resulted in a precipitous drop in the hematocrit, from a normal level around 42 per cent to anemic levels between 15 and 20 per cent. The lowest hematocrit was usually recorded the day after the last bleeding, but an appreciable degree of anemia persisted for several days. A mild anemia usually remained on the 20th day, when the mothers were killed and the young, counted *in situ*, examined for malformations, weighed, and fixed.

Sixty-two pregnant rats were bled total amounts equivalent to 6–7 or more cc per 100 gm of body weight. Of these, 30 (48 per cent) failed to produce offspring. Eighteen (29 per cent) of the mothers died before the 20th day, presumably as a consequence of the anemia. (A higher rate of mortality, approximately 50 per cent, was observed in nonpregnant control females bled comparable amounts.) In the remaining 12 (19 per cent) nonproductive pregnancies, the entire products of conception were resorbed. On the other hand,

32 (52 per cent) of the anemic females carried their pregnancies to the 20th day when living young were removed. The resorption rate among these litters averaged only 9.2 per cent, which compares favorably with a rate of 10.3 per cent observed in several control litters, also subjected to laparotomy during gestation. In only three of the litters was the mean weight of young appreciably less (one standard deviation or more) than the mean of young from control litters. Congenital malformations were noted in 3 of 314 live-born young. This is less than 1 per cent incidence in the whole group and is, in itself, of questionable significance. It is more likely significant that all of the abnormal individuals occurred in litters from mothers bled beginning on the 9th day and that they comprised about 5 per cent of this group. FIGURE 1 shows a typical abnormal animal which exhibits edema, spina bifida, polydactylism and syndactylism. These anomalies have not been observed in several hundred control young of the same strain.

Thus, it appears that hemorrhagic anemia of sufficient severity to terminate pregnancy or kill the mothers in half of the cases, has little effect on the offspring in the remaining pregnancies that continue to term. The rate of mortality of the developing young was not increased and their rate of growth was not decreased by the maternal anemia. A few malformed young were produced but, until larger numbers of offspring are studied, the real significance of this finding is not clear.

Another form of physiologic alteration under study is reduced liver function. Destruction of a large proportion of the liver tissue in pregnant rats has been achieved by the administration of doses of carbon tetrachloride which were sufficient to kill approximately half of nonpregnant rats of comparable weight. This dose consisted of 0.3 cc of carbon tetrachloride by stomach tube or 0.8 cc subcutaneously, administered in corn oil in two or three doses on as many successive days of gestation. To verify pregnancy and to count the embryos, laparotomy was performed on the first day of treatment, which varied between the 7th and the 11th days. Of 29 animals thus treated, somewhat more than half (59 per cent) failed to produce offspring. Only 21 per cent of the pregnant animals died (notably less than in nonpregnant controls given the same dosage), but 38 per cent lost their entire litters as a result of early resorption. Twelve (41 per cent) of the carbon-tetrachloride-treated mothers carried young to the 20th day. The resorption rate among these young was within normal limits (9.1 per cent), none were malformed, and only one litter contained retarded young. Again it is apparent that alterations of maternal physiologic state sufficiently drastic to terminate pregnancy or cause the death of the mother in many instances do not necessarily affect the developing young in the pregnancies which survive the treatment.

A different result was obtained when trypan blue was injected during pregnancy. In order that the data might be more nearly comparable to those from other experiments in this series, the procedure originally outlined by Gillman, Gilbert, and Gillman[3] has not been followed. Instead, the experiment was set up in accordance with our standard procedure in that treatment of the mothers was confined to the period between the 7th and 13th day. Daily injections of 1 cc of 1 per cent solution of trypan blue (in distilled water) were made on three

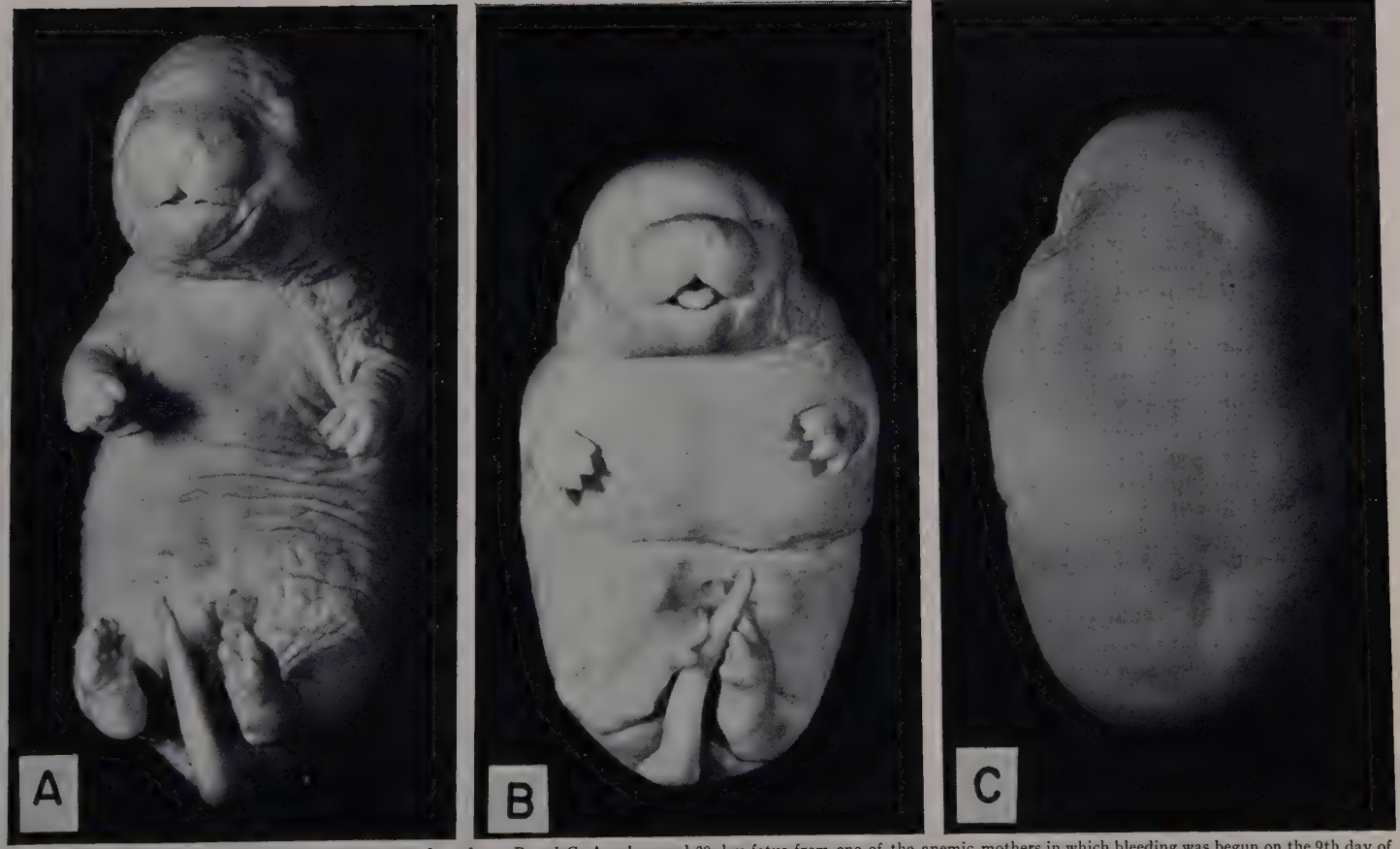

FIGURE 1. A. Normal 20-day fetus from a control mother. B and C. An abnormal 20-day fetus from one of the anemic mothers in which bleeding was begun on the 9th day of pregnancy. In addition to extreme edema, this animal also exhibited polydactylism on both hind feet, syndactylism on the right hind foot (B), and spina bifida (C).

successive days. Laparotomy was performed on the first day of injection. The treatment had relatively little effect on the mothers; of 16 only two died before term and in one, pregnancy was prematurely terminated by complete resorption. Eighty-one per cent of the pregnancies yielded litters containing some living young on the 20th day. Unlike the normal litters from mothers that survived anemia and carbon tetrachloride poisoning, however, these were conspicuously abnormal. The resorption rate between laparotomy and term was 30 per cent, as contrasted with 10.3 per cent for control litters. Of the living young, at least 16 per cent bore externally visible malformations. Several additional animals were suspected of having a moderate degree of hydrocephalus, but verification must await dissection or sectioning. Three litters were retarded in weight, two of them markedly so. Some of the several types of malformations encountered are illustrated in FIGURE 2, which shows a normal animal (A), and an example of sirenomelus (B) in which the hind limbs are fused, the tail is rudimentary, and there are no anal or urogenital openings. The third animal (C) has exencephaly, and the fourth (D) has meningocele and umbilical hernia. Other types observed were hydrocephalus, anencephaly, spina bifida, and anophthalmos. None of these anomalies occurred in young whose mothers were injected beginning on the 11th day. Injections begun on days 7 or 8, however, caused malformation in as many as 30 per cent of the young. It is clear, therefore, that trypan blue injections, which cause relatively moderate alterations in the mothers, are quite deleterious to the embryo at certain periods in development.

Another agent, maternal vitamin A deficiency, has been shown to be even more damaging to the developing embryo.[12] Sixteen mothers exhibited the usual signs of chronic vitamin A deficiency, but there was no mortality. Nevertheless, the deficiency was not compatable with the continuation of pregnancy in 9 of the mothers in which pregnancy was terminated several days before term by complete resorption. Only 7 mothers yielded living offspring when killed at or prior to term. The resorption rate in these litters was estimated to have been 32 per cent, on the basis of embryonic and placental remains, although no laparotomies were performed during early pregnancy in this experiment. The most striking observation was that 78 per cent of the surviving offspring were malformed. The defects, some of which are illustrated in FIGURE 3, largely affected visceral structures: eyes, cardio-vascular system, diaphragm, and urogenital system.

The data from the vitamin A-deficiency experiment has been summarized in TABLE 2 in the same manner as the experiments described above. Less than half of the deficient mothers produced offspring, as was also the case in carbon tetrachloride poisoning. In the latter case, the lost pregnancies were due to maternal death as well as total resorption of litters, but in the vitamin A-deficient mothers complete resorption of litters alone accounted for the nonproductive pregnancies. A similar difference is apparent when the results of vitamin A deficiency are compared with those of anemia, in which there was a relatively high rate of maternal death but a somewhat lower rate of total resorption. The condition of the litters that went to term bears no relation to the prevailing effects of the agent on maternal death or on the continuation of

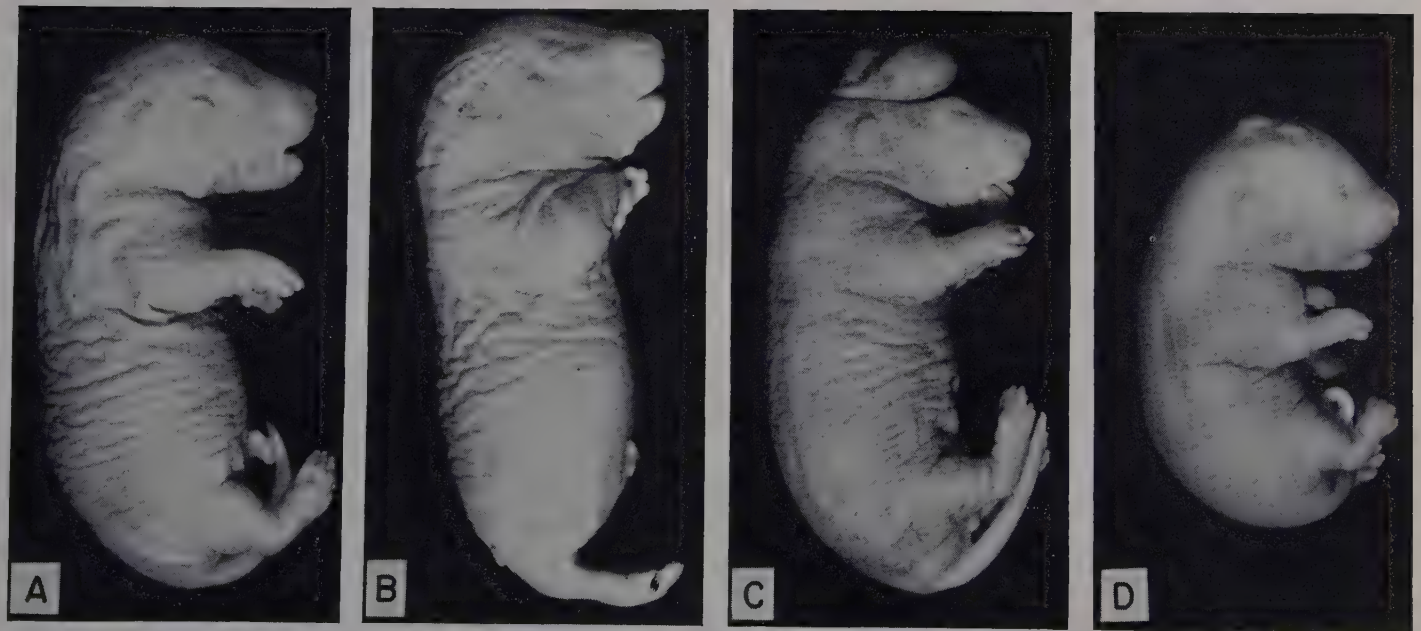

FIGURE 2. A. Normal 20-day fetus from a control mother. B-D. Abnormal 20-day fetuses from mothers injected with trypan blue during pregnancy. The anomalies are sirenomelus (fused hind limbs), rudimentary tail, and absence of anal and urogenital openings (B); exencephaly (C); and meningocele and umbilical hernia (D).

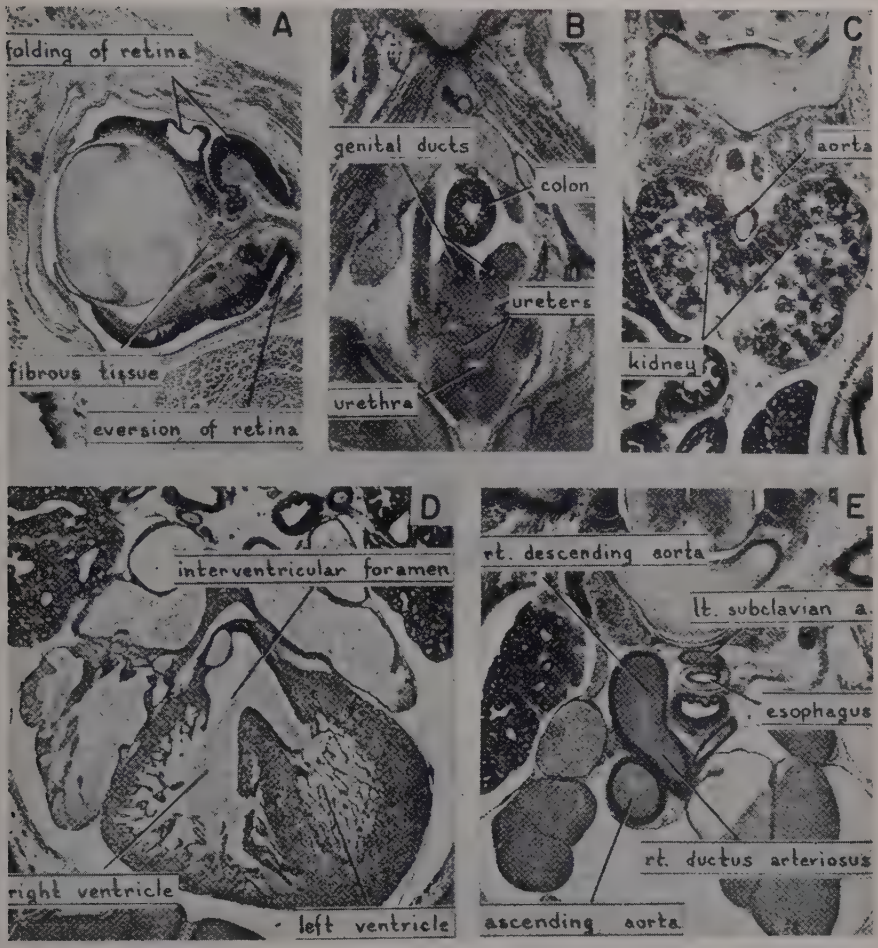

FIGURE 3. Photomicrographs of representative malformed organs from the offspring of vitamin A-deficient mothers. A. Various ocular anomalies including retrolenticular fibroplasia, cysts, and eversion of retina. B. Ectopic opening of ureters into urethra. C. Horseshoe kidney. D. Interventricular septal defect of the heart. E. Right-sided arch of aorta and ductus arteriosus, associated with abnormal left subclavian artery.

pregnancy (TABLE 2). Virtually normal litters were obtained from mothers subjected to anemia or carbon tetrachloride poisoning, despite the fact that both of these agents killed several mothers and caused premature termination of pregnancy in several others. Highly abnormal litters, both as regards the rate of resorption and the incidence of congenital malformations in survivors, were obtained from mothers subjected to trypan blue injections or vitamin A deficiency, neither of which was particularly lethal to the mothers.

The comparative data presented in TABLE 2 justify the following conclusions: (1) All physiologic disturbances during pregnancy are not capable of altering the characteristics of the offspring; (2) Little correlation exists between the

TABLE 2

A COMPARISON OF THE EFFECTS OF FOUR AGENTS ON PREGNANCY AND THE OFFSPRING

Agent	Pregnancy terminated		Pregnancies yielded offspring	Condition of offspring in litters reaching term	
	Mothers died	Entire "litter" resorbed		Prenatal mortality	Survivors malformed
Hemorrhagic anemia	29%	19%	52%	9%	<1%
Carbon tetrachloride poisoning	21	38	41	9	0
Trypan blue injections	13	6	81	30	16
Vitamin A deficiency*	0	56	44	32	78

* Data from Wilson and Barch.[12]

severity of the maternal reaction, as reflected in maternal death or early termination of pregnancy, and the extent to which the offspring may be affected in surviving litters. On this basis it is postulated that those agents capable of acting on or through the pregnant mother to cause maldevelopment of the young must have some specific relation to the needs of the developing embryo. They should not be regarded merely as nonspecific influences that happen to act at a critical time in development.

Summary

(1) A standardized procedure has been employed to test in rats the effects on the developing embryo of several types of physiologic stress in the mother during pregnancy.

(2) Hemorrhagic anemia of such severity as to cause maternal death or early termination of pregnancy in 48 per cent of the mothers, had very little effect on the offspring in litters that reached term.

(3) Carbon tetrachloride poisoning in doses sufficient to cause maternal death or early termination of pregnancy in 59 per cent of mothers had no demonstrable effect on the young in litters that reached term.

(4) Trypan blue injections, on the other hand, only occasionally caused maternal death or termination of pregnancy, but the litters that reached term had high resorption rates and contained 16 per cent of malformed young.

(5) Deficiency of vitamin A, as previously reported, did not cause maternal mortality but resulted in premature termination of pregnancy in 56 per cent. Litters reaching term had high resorption rates and 78 per cent of malformed young.

(6) It was concluded that all forms of physiologic alteration during pregnancy, however severe they may be, are not capable of affecting the characteristics of offspring which go to term.

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THE EFFECTS ON DEVELOPMENT WHEN EGGS AND SPERM ARE AGED BEFORE FERTILIZATION

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Developmental defects which result from the overripening of gametes before fertilization have been studied in greatest detail in the invertebrates and lower vertebrates. The eggs of fish and amphibia have been particularly useful in experimental investigations of this type because of their ready availability, the large number of gametes that may be obtained from a single animal, and the relative ease with which they may be handled and observed in the laboratory.[1, 2, 3]

Witschi[2] describes a method whereby frogs were induced to retain their mature eggs within the uteri for varying intervals of time by separating the females from the males and keeping them in dry containers at room temperature. By subsequently removing the eggs, either by laparotomy or stripping, the aging gametes could be fertilized by artificial insemination and their development followed. He found that fertilization and development remained relatively normal in eggs which had been retained for 72 to 96 hours. For the next several days the eggs became overripe gradually and either failed to be fertilized or, if penetrated by a sperm, developed abnormally. After approximately one week all retained eggs became unfertilizable.

The aging eggs of the amphibia gradually lose their vitality. Eggs that are affected slightly by the aging process show only transitory retardations in the rate of development. On the other hand, highly overripe eggs may not develop beyond the earliest cleavage stages.

Some of the developmental abnormalities encountered as the result of overripeness of the eggs in amphibians may be listed as follows:

(1) Tendency to produce axial duplications, especially in the regions of the head. These may take the form of twins either of equal size and normal appearance, or of unequal dimensions and consisting primarily of teratomatous swellings.

(2) Polymelia and polydactyly.

(3) Deficiencies in organogenesis leading especially to acephaly and microcephaly.

(4) Failure of the normal differentiation of various tissues and organ systems.

The observations of Witschi are particularly significant in that he followed the developmental defects beyond the stage of metamorphosis and established clearly that "delayed fertilization acts as a teratogenic factor throughout the order of the anuran amphibians."

The investigations of Mišić[1, 3] on the aging egg of the rainbow trout similarly established the deleterious effect of overripeness before fertilization. Not only was there an increase in mortality in the aging gametes that were successfully fertilized, but there was also a significant increase in the percentage of mal-

formations, especially those involving the eyes and reproductive organs. Various defects of partial twinning were particularly common.

The teratogenic effect of aging eggs before fertilization has been observed also in the lamprey,[4] the sea urchin,[5] the marine annelid, *Hydroides hexagonia*,[6] and others.

Gamete Age and Development in the Mammals

As studies on the physiology and biochemistry of the gametes of mammals are pursued, it is becoming evident that, unless the sperm penetrates the egg within a short interval of time after ovulation and insemination, rapid degeneration of the sex products occurs. In domestic animals the reproductive processes are so timed that spermatozoa have reached the site of fertilization and are ready to penetrate into the egg within a few minutes after it has been shed from the follicle.

It is well established that during the follicular phase of the reproductive cycle a considerably larger number of ovarian follicles develop than will ovulate. Many of these degenerate before the stage of preovulatory swelling is reached. A few, however, will continue to enlarge and both follicle and oöcyte may undergo all of the preovulatory changes with the exception of the loosening of the cumulus. The eventual fate of these follicles is atresia.

It has been suggested that some of the ova shed at the time of ovulation may not have attained the necessary maturity and as a result may either fail to be fertilized, or if penetrated by a sperm, develop abnormally.

There is much evidence that, in both domestic mammals[7, 8] and man,[9, 10] at least thirty per cent of the ovulated eggs either fail to be fertilized or produce abnormal embryos which die during the early stages of pregnancy. It is usually assumed that the egg is inherently responsible for the failure of fertilization. However, as yet there are no criteria for recognizing a healthy, viable egg—one that may be fertilized and develop normally. The biologic basis for infertility in relation to the egg and sperm remains largely unexplored.

It is important to note also that sperm penetration into the egg is a very complex process. After a spermatozoon has reached the site of fertilization it must overcome the barriers of the granulosa cells, the zona pellucida, and the vitellus itself. Observations on the rabbit[11] and rat[12] have shown that this is not invariably accomplished in spite of the presence of an adequate number of spermatozoa at the site of fertilization.

The vitality of sperm stored in the ductus deferens is also of importance in relation to the problem of the fertilizing capacity of the gametes. Undoubtedly millions of sperm of varying maturity are inseminated at the time of ejaculation. It would be important to know whether any of the immature or overripe spermatozoa are capable of ascending the female reproductive tract, penetrate the egg and effect teratogenic development. The limited evidence at hand indicates that spermatozoa of lowered vitality do not reach the site of fertilization. Of an average of fifty million spermatozoa, which were inseminated into the uteri of rats, only five to ten could be recovered from the ovarian portion of each oviduct twelve hours after insemination.[13] In the rabbit only a few to 1000 spermatozoa are ever found in the ovarian segment of the oviduct where

fertilization occurs.[14] The small numbers of sperm which successfully reach the site of fertilization probably represent those which are the most physiologically fit.

Spermatozoa may retain their fertilizing capacity within the oviducts of the rat for a maximum of 14 hours,[15] in the guinea pig 22 hours,[16] in the mouse 6 hours,[17] and in the rabbit 30 hours.[18] It has been demonstrated conclusively, in both the rat and guinea pig, that aging of the spermatozoon in the female reproductive tract may affect its ability to penetrate the egg. If the aged spermatozoon, however, has retained sufficient vitality to penetrate the egg, pronuclear changes and syngamy takes place and the ovum develops normally. Apparently an "all or none" phenomenon exists in regard to the ability of the aged sperm to penetrate the egg. Whether the sudden loss of the ability of the aged spermatozoa to fertilize ova will be found in other animals besides the rat and guinea pig is still to be determined.

The fertilizable life of the mammalian ovum has been experimentally determined in only a few rodents, TABLE 1.

The significance of studies of this type is related directly to the accuracy with which the time of ovulation and semination may be determined.[12]

In the rat and guinea pig, the onset of the heat response has been shown to be a reliable and accurate method for the determination of the time of ovulation. In the rabbit, ovulation may be determined accurately from the time of mating. In each of these animals, as the time of artificial insemination is successively advanced after ovulation, there is a progressive decrease in the number of eggs fertilized and a striking increase in the number of abnormally developing ova.[11, 19, 20]

If the overripe eggs of the rat are examined during the period of pronuclei formation and first segmentation division, a number of alterations in their development may be observed.[12] In the first place, thirty per cent of the eggs which have been aged 9 to 12 hours before insemination failed to be penetrated by spermatozoa despite the presence of an adequate number to accomplish

TABLE 1
THE FERTILIZABLE LIFE OF THE MAMMALIAN OVUM

Animal	Length of fertilizable life	Investigator
Opossum	Morphological signs of degeneration appear within 24 hours after ovulation	Hartman (1919)
Mouse	a) 12 hours. Matings 13 hours after ovulation results in reduced fertility	Long (1912)
	b) 6 hours—estimation	Lewis & Wright (1935)
Rat	>12 hours—experimental	Blandau & Jordan (1941)
Guinea pig	>20 hours—experimental	Blandau & Young (1939)
Ferret	>30 hours—experimental	Hammond & Walton (1934)
Rabbit	6 hours—experimental	Hammond (1934)
Sheep	24 hours—estimation	Green & Winters (1935)
Mare	Short	Day (1940)
Monkey	23 hours—estimation	Lewis & Hartman (1941)

semination. In the control inseminations less than five per cent of the eggs failed to be fertilized. It is speculated that, as aging progresses, some physical or chemical change occurs within the egg or its membranes so that sperm penetration is not accomplished.[12] Over forty per cent of the eggs which were successfully penetrated by spermatozoa in this group showed some abnormality in pronuclear development. In some aged eggs only the male pronucleus developed normally. The female chromosomal mass either failed to differentiate at all, or underwent partial development only. Fragmentation, or the formation of accessory nuclear components, was frequently observed in this group. In some ova, pronuclear development progressed normally until the time of cleavage when nuclear fragmentation began.

Even though seventy per cent of the greatly overripe rat eggs may be penetrated by sperm, various abnormalities of development may result which are not compatible with continued growth. Thus at the time of implantation only four per cent of the experimental rats are impregnated. Furthermore, the ova which successfully implant are retarded in their development and the majority of these die before the fetal period is reached.

There is a striking correlation in the results obtained in the investigations on the aging egg of the rat with those of similar observations on delayed fertilization in the guinea pig. The fertilizable life of the rat egg is approximately one half that found for the guinea pig.[19] In the guinea pig the first effects of overripeness were seen in embryos from females inseminated 8 hours after ovulation. No normal development followed inseminations more than 20 hours after ovulation. As far as could be determined from these investigations, the principal effects of aging were the early death of the ovum in the preimplantation period and retardation in the rate of development in the ova which had implanted.

In summary, overripeness of the eggs of amphibia and fish before fertilization leads to teratogenic development in which a wide variety of developmental abnormalities are produced. These abnormalities are manifest, not only during the various stages of development, but also after hatching. In several mammals so far studied, there is a gradual reduction in the fertilizable life of the ovum as the period between ovulation and insemination is prolonged. The effects of overripeness on development may be manifest during the period of pronuclei formation and syngamy. After implantation, the overripe egg may be retarded in its development or may die and be resorbed or aborted before the fetal period is reached. Whether moderate overripeness of the mammalian egg leads to definite teratogenic development in the fetal and postnatal period is as yet unexplored.

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THE EFFECTS OF THE AGE OF THE MOTHER ON THE SEX RATIO AT BIRTH IN JAPAN

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It has been stated by some investigators (Bidder,[1] Copeman and Parsons,[2] Parkes,[3] King and Stotsenburg,[4] Bonnier,[5] Ciocco,[6] *etc.*) that the age of the mother has a pronounced influence on the sex of her young.

We tried to find such effects of the age of the mother on sex ratio of births in the Japanese population, using official vital statistics and other Japanese data.

1. Data from Japanese Vital Statistics from 1937 through 1943

The number of births and stillbirths in terms of mothers' age (classified in 5-year groups), based on vital statistics from 1937 through 1943, is shown in TABLE 1 and FIGURE 1.

The number of births of the age class of mothers under 14 is very small and we may omit it in considering this problem. The yearly sex ratios (boys to 100 girls) at birth are not always the same, but the curve of sex ratio of birth traced by mothers' age class shows almost the same tendency every year. Except for 1940, the sex ratio was found to be mostly between 105 and 107 when the mothers are under 19. Even including the exception, 1940, the difference between the ratio for mothers under 19 and those 20–24 is not significant. The ratio gradually falls as the mothers' age increases until it is about 104 when the mothers are 35–44. The bottom of the curve always occurs in the same age group. It is reached mostly when mothers are in the 35–39 or 40–44 age class. The curve becomes comparatively variable after the 45–49 age class, because then the absolute number of births becomes small (under ten thousand per year). But the curve for the average of the seven years rises significantly when the mothers are over 50.

Averaging those seven years' data, the differences of the sex ratio at birth between the age classes of mothers 15–24 and 35–44, and also between the age classes of mothers 35–44 and over 50, are significant.

The sex ratio of stillbirths in Japan is generally higher than that of live births; during those years the sex ratio of stillbirths was about 120. The stillbirth ratio is different by mothers' age, and is about the same in each years' data. When the mothers' age is 15–19 and 40–49 the stillbirth ratio is 6.0–7.0 per 100 births, and when the mothers age is 20–34 and over 50 it is about 4.0 or lower.

Regrettably, the vital statistics of 1937–43 did not indicate the number of each sex of stillbirths by mothers' age classes. Assuming that the sex ratio of stillbirths is not different by mothers' ages and is constant, distribute the number of males and females in stillbirths respectively to number of births of every mothers' age class, and we get the estimated sex ratio of all births including stillbirths by every age class of mothers, as the dotted line of Figure 1 shows.

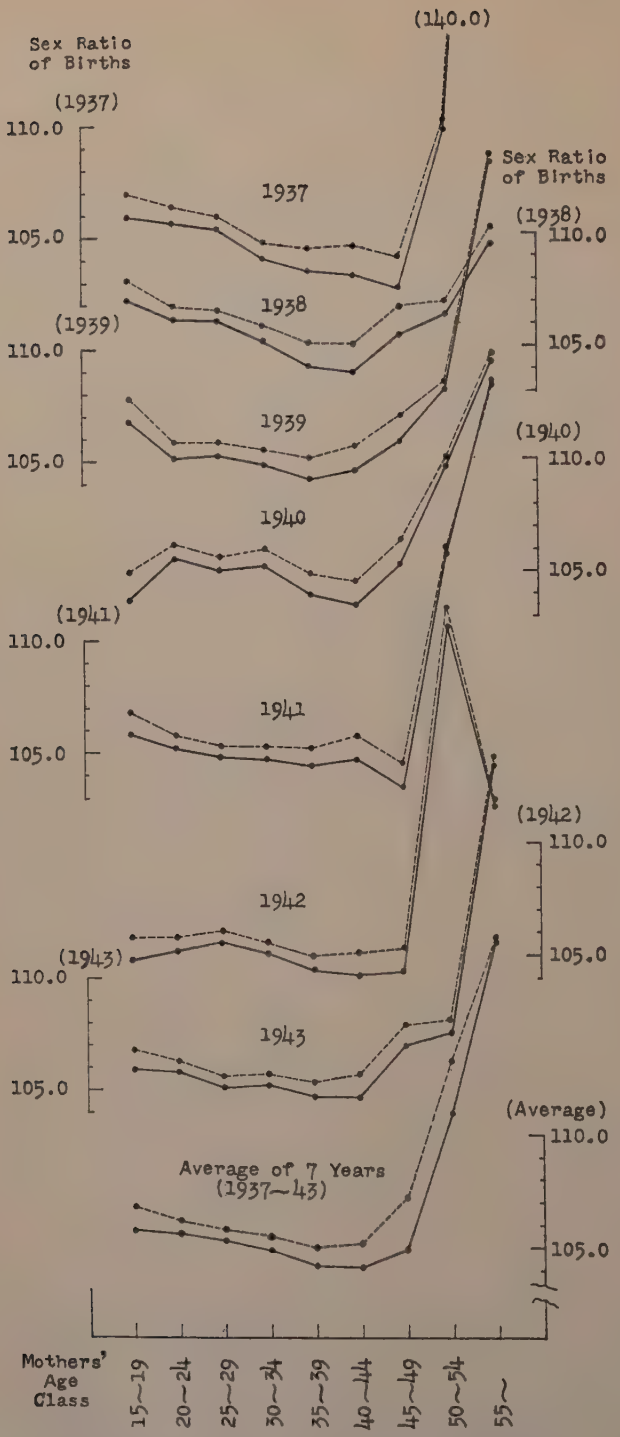

FIGURE 1. Sex ratio of live births (solid line) and of all births (dotted line), including stillbirths, by mothers' age class, from 1937 through 1943 in Japan.

2. Data from Japanese Vital Statistics from 1947 through 1950

Japanese vital statistics were not published from 1944 to 1946, about the end of the World War II. Also, after the war, family planning increased in Japan, and the number of births to older mothers became smaller yearly. But in the data from Japanese vital statistics of the four years 1947 through 1950 (see FIGURE 2, TABLE 2), the same, but less pronounced, curve of the sex ratio of births in terms of the mothers' age class is found that we found in the data of 1937–43.

In the curve of the sex ratio by the mothers' age class in these data, we can find some variations in each year. But the curve of an average of the four years shows the same tendency as in FIGURE 1. The sex ratio of births to young mothers is higher, becomes lower as the mothers' age increases, but is highest for the group over 49.

From these data we can also get the sex ratio of stillbirths by mothers' age class. The dotted line of FIGURE 2 shows the sex ratio of all births including stillbirths of 5 months or more gestation. The dotted line runs fairly parallel to the solid line, except that for mothers over 50 the difference becomes larger, which is a little different from the data of 1937–42.

We can get the sex ratio of live births, stillbirths, and live plus stillbirths by mothers' age class, and by order of birth, from these data, as shown in TABLES 3–5. The sex ratio of live births by birth order shown in FIGURE 3 has some likeness to the sex ratio of live births by mothers' age class (except for small variations,) but it is less pronounced. No other remarkable characteristic of the sex ratio of live births and live plus stillbirths, by order of births, can be deduced from TABLES 3–5.

3. Data from Another Source in Japan

Professor Komatsu, of Wakayama University School of Medicine, proved a tendency similar to that which we found, in the relation between age of mother and sex ratio (reported at the fourth meeting of the Japanese Public Health Association in Tokyo in October 1951). He based his report upon the data of 2,674 families, which he gathered, using high school boys and girls as informants about their families. He classified mothers in three groups: married under 20; from 21 to 25; and over 26. And he tested the sex ratio of children following their birth order. FIGURE 4 shows the result, namely, that the sex ratio of the first born by mothers married youngest (under 20) is the highest. The sex ratio of the first born by mothers married next youngest (21–26) is nearly that of the second born by mothers married youngest. Thus the sex ratio of the born diminishes according to the age of mother rather than to the order of births. And it seems the average of the sex ratio rises again in the oldest age group of mothers.

4. Sex Ratio of Births and the Fathers' Age

Concerning the sex ratio of births in relation to the fathers' age we only have data for 1942. The curve of the sex ratio is similar to that of the mothers' age classes, as we see in FIGURE 5. But the rise of the curve after 50–54 is less than that of mothers.

TABLE 1

AGE CLASS OF THE MOTHERS AND THE SEX RATIO OF CHILDREN BORN DURING 7 YEARS (1937–43) IN JAPAN

Age class of mothers	Sex of the children	1937	1938	1939	1940	1941	1942	1943	Total of 7 years
Under 14	Boys	66	75	106	146	16	15	15	439
	Girls	60	77	133	152	13	14	13	462
15–19	Boys	31,799	27,367	23,891	22,959	16,279	15,572	14,649	152,516
	Girls	30,004	25,525	22,362	22,109	15,384	14,846	13,828	144,058
	Sex ratio	105.98	107.21	106.84	103.84	105.81	104.89	105.93	105.87
20–24	Boys	266,515	228,729	207,001	221,976	217,286	210,248	209,954	1,561,709
	Girls	252,177	215,119	196,806	209,842	206,463	199,679	198,344	1,478,430
	Sex ratio	105.68	106.32	105.18	105.78	105.24	105.29	105.85	105.63
25–29	Boys	332,117	294,371	296,147	335,994	360,073	343,166	336,612	2,298,480
	Girls	315,217	277,167	281,457	319,494	343,569	324,707	320,092	2,181,703
	Sex ratio	105.36	106.20	105.22	105.16	104.80	105.68	105.15	105.35
30–34	Boys	229,706	206,670	215,699	253,164	288,858	284,932	296,053	1,775,082
	Girls	220,817	196,133	205,675	240,234	275,800	271,056	281,313	1,691,028
	Sex ratio	104.02	105.37	104.87	105.38	104.73	105.11	105.23	104.97
35–39	Boys	155,056	142,274	143,227	156,422	170,139	175,593	187,469	1,130,180
	Girls	149,906	136,448	137,471	150,373	162,813	168,278	179,024	1,084,313
	Sex ratio	103.43	104.26	104.19	104.02	104.49	104.34	104.71	104.23

TABLE 1 (CONTINUED)

40–44	Boys	55,887	53,056	53,068	56,871	72,319	75,874	78,810	445,885
	Girls	54,111	51,037	50,779	54,945	69,072	72,824	75,206	427,974
	Sex ratio	103.28	103.95	104.51	103.50	104.70	104.18	104.79	104.18
45–49	Boys	5,825	5,260	5,213	5,763	7,745	8,296	8,866	46,968
	Girls	5,676	4,982	4,927	5,471	7,487	7,951	8,280	44,774
	Sex ratio	102.62	105.58	105.80	105.33	103.44	104.34	107.07	104.90
50–54	Boys	928	974	1,007	1,172	1,281	1,255	1,040	7,657
	Girls	846	915	931	1,068	1,125	1,049	967	6,901
	Sex ratio	109.69	106.44	108.16	109.73	113.86	119.63	107.54	110.95
55 and over	Boys	466	465	447	462	487	432	385	3,144
	Girls	332	424	378	404	401	387	322	2,648
	Sex ratio	140.36	109.66	118.25	114.35	121.44	111.62	119.56	118.73
Total	Boys	1,078,425	959,241	945,806	1,054,929	1,134,483	1,115,393	1,133,853	7,422,060
	Girls	1,029,146	907,827	900,919	1,004,092	1,082,127	1,060,790	1,077,376	7,062,291
	Sex ratio	104.78	105.66	104.98	105.06	104.83	105.14	105.24	105.09

TABLE 2

AGE CLASS OF MOTHERS AND SEX RATIO OF LIVE BIRTHS AND OF STILLBIRTHS (OF GESTATION 5 MONTHS OR OVER) DURING 4 YEARS (1947–50) IN JAPAN

Age class of mothers	Sex and sex ratio	Number and sex ratio of live births				Number and sex ratio of stillbirths			
		1947	1948	1949	1950	1947	1948	1949	1950
Under 14 years	Boys	28	22	20	28	3	12	22	39
	Girls	23	30	23	21	7	13	21	33
15–19	Boys	31,547	37,633	34,566	28,934	1,947	2,660	3,744	4,193
	Girls	29,625	35,444	32,778	27,382	1,680	2,251	3,108	3,594
	Sex ratio	106.49	106.18	105.45	105.67	115.89	118.17	120.46	116.67
20–24	Boys	316,774	353,972	355,233	322,308	13,850	17,019	19,954	21,319
	Girls	298,884	333,933	339,834	302,489	11,482	13,874	16,560	17,764
	Sex ratio	105.99	106.00	104.53	106.55	120.62	122.67	120.50	120.01
25–29	Boys	426,118	420,349	450,966	408,623	14,932	16,669	20,471	21,135
	Girls	400,483	397,561	430,593	385,618	12,622	13,977	17,329	17,781
	Sex ratio	106.40	105.73	104.73	105.97	118.30	119.26	118.13	118.86
30–34	Boys	331,454	301,114	299,535	255,039	11,818	12,088	14,502	14,965
	Girls	313,875	284,476	284,505	241,201	10,029	10,338	12,239	12,719
	Sex ratio	105.60	105.85	105.28	105.74	117.84	116.93	118.49	117.66
35–39	Boys	204,370	196,694	180,713	143,280	9,492	9,858	11,556	11,339
	Girls	194,615	186,392	173,217	135,501	8,087	8,419	9,917	9,634
	Sex ratio	105.01	105.53	104.33	105.74	117.37	117.09	116.53	117.70
40–44	Boys	59,948	63,662	55,444	42,160	4,037	4,575	5,184	4,989
	Girls	57,858	60,511	52,325	39,793	3,474	3,940	4,502	4,452
	Sex ratio	103.61	105.21	105.96	105.95	116.21	116.12	115.15	112.06
45–49	Boys	5,086	4,537	3,244	2,257	423	523	518	476
	Girls	4,883	4,151	3,114	1,956	372	441	444	397
	Sex ratio	104.16	109.30	104.17	115.39	113.71	118.59	116.67	119.90
50 years and over	Boys	1,019	535	242	168	55	46	35	80
	Girls	911	509	208	143	49	38	30	24
	Sex ratio	111.86	105.11	116.35	117.48				
Total	Boys	1,376,986	1,378,564	1,380,008	1,203,111	56,675	63,571	76,059	78,604
	Girls	1,301,806	1,303,060	1,316,630	1,134,396	47,866	53,391	64,222	66,450
	Sex ratio	105.78	105.79	104.81	106.06	118.40	119.07	118.43	118.29

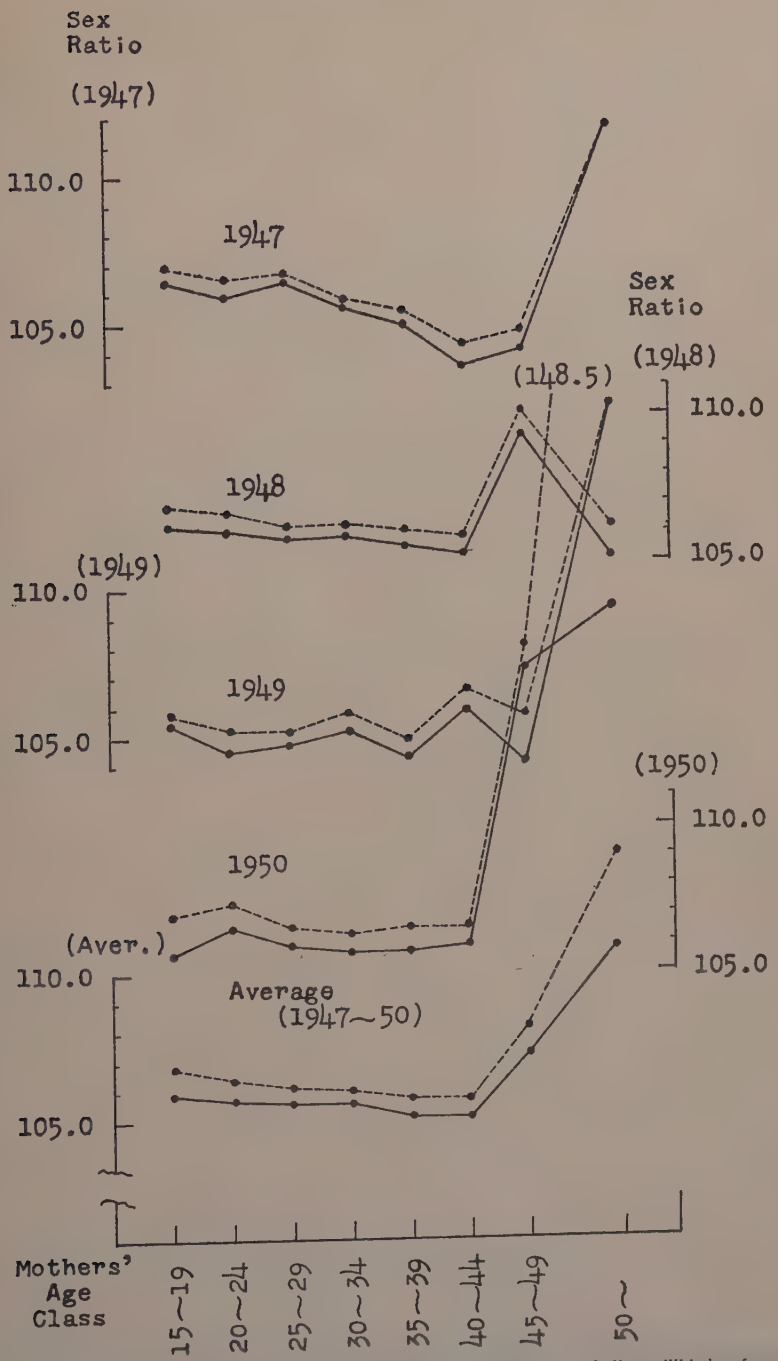

FIGURE 2. Sex ratio of live births (solid line) and of all births (dotted line), including stillbirths of gestation five months or over, by mothers' age class, 1947 through 1950, in Japan.

5. Data on Offspring of Studhorses

On the sex ratio of the offspring of studhorses we were able to get data from the Oou Branch of the Japanese National Stallion Pasture. TABLE 6 shows the sex ratio of 20,443 offspring of 53 stallions by age group. The tendency of the sex ratio to diminish according to the increase in age group of stallions, is recognizable by χ^2-test ($\chi^2 = 9.216$, $n = 3$, $0.05 > P > 0.02$).

Discussion

Such tendencies as we get from Japanese data in the effects of the age of mothers on the sex ratio of their young had already been found by many research workers, but almost all reports seemed to have too few data to justify definite conclusions. Our data on the Japanese population seems to be large enough to give significance to those tendencies.

There is much information available on the relation between sex ratio and age of mothers in various countries. The data agree reasonably well, in general character, with the Japanese data, except for the two oldest age groups. These are completely out of line with those of citizens of Occidental countries, where contraception has been practiced for some time and especially where women approaching older age do not want parturition. For such reasons the Occidental data on parturition for those over 45 is much less. Such data, therefore, on sex ratio of older mothers' births are not yet known. Contrary to this, contraception was formerly not prevalent in Japan except among a few intelligent people, and, especially in the era from 1937 through 1943, when the government encouraged the nation to have children although, since the end of the war, contraception has been gradually increasing in Japan among the general public. Therefore, in Japan, hindrances to natural fecundation should have been less than in other countries.

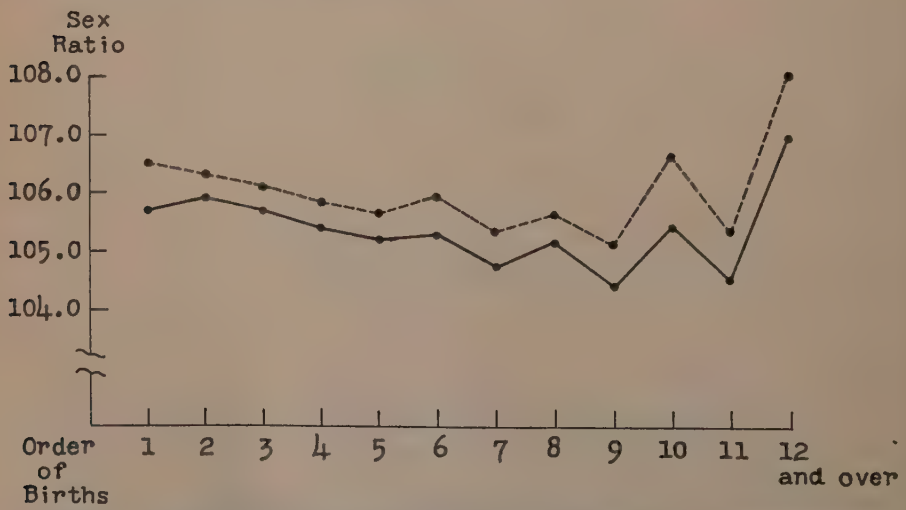

FIGURE 3. Sex ratio of live births (solid line) and of live and stillbirths after five or more months gestation (dotted line) by order of births, from the data of 1947–50.

On the basis of 1947–49 Japanese live birth statistics, Maruoka[7] reported that the sex ratio is extraordinarily high for the twelfth or subsequent births. He supposed that this was due to careless nursing of late girl babies by mothers already having many children, and to their being reported as stillbirths if they die. But a mistake was found in his calculation. Furthermore, his supposition is denied by our data, the sex ratio of stillbirths of 5 months or more gestation is not low for high order births.

Concerning the fact that the sex ratio among first children is highest, this is probably due to the mothers' youth. It seems Komatsu's data support this explanation. Following Bidder's data, also, we cannot recognize any significant differences of sex ratio at birth, between primipara and multipara (see TABLE 7). In Japan we found parturition by aged mothers (over 50), but not only in the case of aged primipara. The percentage of first children in the parturition by aged mothers is not so high.

It may be supposed that some old mothers claim the babies of unmarried daughters as their own children, as M. Segi pointed out to me. And it is true that the sex ratio of births of illegitimate children (92.9 $\pm$ 0.39 in 1937–43) is far lower than that of legitimate (105.3 $\pm$ 0.06), in Japan. But we cannot know how many per cent of births to older mothers can be the result of such incorrect records, and whether they claim more male babies than female as their own children.

Conditions closely paralleling these for Japanese statistics are also found in some kinds of mammals. Data given by Copeman and Parsons[2] from their inbreeding experiments with mice agree with those for man in that they show that the sex ratio of offspring is at its lowest point when the mother is at the height of her reproductive power, and that when mothers become older the ratio rises (TABLE 8).

In the data of King and Stotsenburg[4] (in which 75 litters totalling 516 young cast by 21 stock albino rats were investigated), the sex ratio becomes lower according to the age of mothers. The observation was continued until the mothers were about seven to nine months old. But the female albino rat, if she is in good physical condition, will continue to bear young until she is about fifteen months old, as the authors said. Therefore it is not unreasonable to consider that, if they had been observed over a longer period, it might have been found that the sex ratio of later offspring was higher, as in the data of Copeman and Parsons. Although these data are too small for definite conclusions, we can recognize a tendency similar to that which we find in the effects of the age of human mothers on the sex ratio at birth (TABLE 9).

Pearl and Parshley[8] once reported that cows have a lower sex ratio of birth in the beginning of puberty than in later periods, though it was found that the result was not statistically significant.

Also, Bidder recognized the same tendency in his compilation of 11,871 births for those born to women of various ages, though the sex ratio of his data is fairly high through all age classes of mothers (TABLE 10).

Bonnier[5] concluded from his statistics of the observation of births in Sweden in 1919, that the sex ratio at the time of conception might be higher the older the mothers' age; but, at the same time, the stillbirths also increased according

TABLE 3

SEX RATIO OF LIVE BIRTHS BY AGE OF MOTHER AND ORDER OF BIRTH THROUGH 4 YEARS (1947–50) IN ALL JAPAN

Birth order	Sex and sex ratio	Total	Age of mother (5 year groups)										
			Under 15 years	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55 years and over	Age unknown
1	Male	1,546,139	98	121,033	871,098	438,613	87,176	22,954	4,254	457	126	41	289
	Female	1,462,661	97	114,243	824,391	414,551	82,457	21,900	4,035	472	107	37	291
	M:F	105.71		105.95	105.67	105.81	105.72	104.43	105.43	(96.82)	(117.76)		(99.31)
2	Male	1,221,221		11,040	388,164	606,129	170,316	38,634	6,058	501	114	37	228
	Female	1,153,247		10,422	366,535	572,477	161,230	35,882	5,866	462	111	38	224
	M:F	105.89		105.93	105.90	105.88	105.64	107.67	102.92	(108.44)	(102.70)		(101.79)
3	Male	830,725		542	74,392	413,240	266,746	65,494	9,454	558	99	41	159
	Female	785,641		497	70,366	391,427	251,062	62,682	8,804	547	79	27	150
	M:F	105.74		(109.05)	105.72	105.57	106.25	104.49	107.38	(102.01)	(125.32)		(106.00)
4	Male	585,004		30	11,471	175,319	280,378	102,534	14,287	725	117	33	110
	Female	554,945		37	10,892	166,620	266,004	96,694	13,752	687	92	43	124
	M:F	105.42			105.32	105.22	105.40	106.04	103.89	(105.53)	(127.17)		(88.71)
5	Male	407,891		2	2,000	53,880	200,292	128,900	21,487	1,072	132	34	92
	Female	387,669		1	1,824	51,222	189,678	123,323	20,424	972	105	31	89
	M:F	105.22			109.65	105.19	105.60	104.52	105.20	110.29	(125.71)		
6	Male	285,526			494	13,854	109,563	130,476	29,475	1,424	156	42	42
	Female	271,122			508	13,311	104,072	123,737	27,970	1,283	154	40	47
	M:F	105.31			(97.22)	104.08	105.28	105.45	105.38	115.02	(101.30)		
7	Male	193,693			155	3,353	47,821	104,994	35,275	1,819	174	60	42
	Female	184,916			163	3,122	45,750	100,294	33,723	1,607	179	42	36
	M:F	104.75			(95.09)	107.40	104.53	104.69	104.60	113.19	(97.21)		
8	Male	125,178			56	794	17,080	69,383	35,529	2,073	192	39	32
	Female	119,034			51	734	16,403	66,110	33,565	1,951	165	35	20
	M:F	105.16				(108.17)	104.13	104.95	105.85	106.25	(116.36)		

TABLE 3 (CONTINUED)

9	Male	73,366			6	202	5,404	36,661	28,766	2,121	147	44	15
	Female	70,286			3	160	5,078	35,288	27,533	2,034	142	38	10
	M:F	104.38				(126.25)	106.42	103.89	104.48	104.28	(103.52)		
10	Male	38,831				57	1,462	16,134	19,212	1,810	113	33	10
	Female	36,823				56	1,400	15,252	18,245	1,722	108	28	12
	M:F	105.45					104.43	105.78	105.30	105.11	(104.63)		
11	Male	17,734				3	393	5,887	10,088	1,270	70	19	4
	Female	16,985				6	359	5,632	9,781	1,130	65	8	4
	M:F	104.41					(109.47)	104.53	103.14	112.39			
12 & over	Male	11,553					150	2,767	7,246	1,288	76	23	3
	Female	10,802				3	127	2,639	6,707	1,228	77	20	1
	M:F	106.95					(118.11)	104.85	108.04	104.89			
Order unknown	Male	1,808		33	451	612	361	239	83	6	2	—	21
	Female	1,761		29	406	566	437	213	82	9	—	—	19
Total	Male	5,338,669	98	132,680	1,348,287	1,706,056	1,187,142	725,057	221,214	15,124	1,518	446	1,047
	Female	5,055,892	97	125,229	1,275,139	1,614,255	1,124,057	689,726	210,487	14,104	1,384	387	1,027
	M:F	105.593		105.949	105.736	105.686	105.612	105.122	105.096	107.231	(109.68)	(115.25)	
											110.897		

TABLE 4

SEX RATIO OF STILLBIRTHS (GESTATION 5 MONTHS OR OVER) BY AGE OF MOTHER AND ORDER OF BIRTH THROUGH 4 YEARS (1947–50) IN ALL JAPAN

Birth order	Sex and sex ratio	Total	Age of mother (5 year groups)									
			Under 15 years	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50 years and over	Age unknown
1	Male	96,043	76	11,129	48,438	25,772	7,072	2,624	663	78	26	165
	Female	79,073	74	9,471	39,671	21,196	5,746	2,145	558	69	24	119
	M:F	121.46		117.51	122.10	121.59	123.08	122.33	(118.82)			
2	Male	49,567		1,295	17,736	20,709	6,949	2,277	493	52	13	43
	Female	42,054		1,025	14,904	17,543	5,991	2,062	444	39	6	40
	M:F	117.87		(126.34)	119.00	118.05	115.99	110.43	(111.04)			
3	Male	33,093		105	4,667	14,581	9,485	3,404	750	56	12	33
	Female	28,372		114	3,947	12,693	8,090	2,841	609	48	6	24
	M:F	116.64			118.24	114.87	117.24	119.82	(108.70)			
4	Male	25,614		10	978	7,672	10,755	4,965	1,117	79	16	22
	Female	21,773		15	865	6,509	8,997	4,321	964	80	10	12
	M:F	117.64			(113.06)	117.87	119.54	114.90	115.87			
5	Male	20,148			210	3,019	8,735	6,424	1,603	114	24	19
	Female	17,426		5	185	2,599	7,521	5,533	1,461	102	8	12
	M:F	115.62			(113.51)	116.16	116.14	116.10	109.72	(111.76)		
6	Male	16,524			73	987	5,642	7,169	2,448	177	19	9
	Female	13,912			59	788	4,795	6,069	2,036	136	18	11
	M:F	118.78				(125.25)	117.66	108.47	120.24	(130.15)		
7	Male	12,469			12	307	2,797	6,230	2,848	237	27	11
	Female	10,751			21	238	2,505	5,294	2,460	209	17	7
	M:F	115.98				(128.99)	111.66	117.68	115.77	(113.40)		
8	Male	8,952			8	83	1,193	4,405	2,934	291	31	7
	Female	7,916			2	88	1,099	3,818	2,605	277	19	8
	M:F	113.09					108.55	132.76	112.63	(105.05)		

TABLE 4 (CONTINUED)

9	Male	5,842			2	44	467	2,574	2,435	302	15	3
	Female	5,069			3	22	364	2,220	2,195	251	11	3
	M:F	115.25					(128.30)	115.95	110.93	(120.32)		
10	Male	3,483			2	9	180	1,278	1,760	237	15	2
	Female	2,860				11	147	1,046	1,452	195	9	—
	M:F	121.78					(122.45)	122.18	121.21	(121.54)		
11	Male	1,744					61	527	983	165	7	1
	Female	1,507				1	45	433	895	126	7	—
	M:F	115.73						(121.71)	(109.83)	(130.95)		
12 & over	Male	1,295					26	358	748	151	10	2
	Female	1,096				3	15	264	687	121	6	—
	M:F	118.16						(135.61)	(108.88)	(124.79)		
Order unknown	Male	135		5	16	24	11	10	3	1	1	64
	Female	120		3	23	18	10	11	2	1	—	52
Total	Male	274,909	76	12,544	72,142	73,207	53,373	42,245	18,785	1,940	216	381
	Female	231,929	74	10,633	59,680	61,709	45,325	36,057	16,368	1,654	141	288
	M:F	118.531		117.972	120.881	118.632	117.756	117.161	114.766	117.291	153.191	132.291

TABLE 5

SEX RATIO OF LIVE AND STILLBIRTHS (GESTATION 5 MONTHS OR OVER) BY AGE OF MOTHER AND ORDER OF BIRTH THROUGH 4 YEARS (1947–50) IN ALL JAPAN

Birth order	Sex and sex ratio	Total	Age of mother (5 year groups)									
			Under 15 years	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50 years and over	Age unknown
1	Male	1,642,182	174	132,162	919,536	464,385	94,248	25,578	4,917	535	193	454
	Female	1,541,734	171	123,714	864,062	435,747	88,203	24,125	4,593	541	168	410
	M:F	106.52	(101.8)	106.83	106.42	106.57	106.85	106.02	107.05	(98.9)	(114.9)	(110.7)
2	Male	1,270,788		12,335	405,900	626,838	177,265	40,911	6,551	553	164	271
	Female	1,195,301		11,447	381,439	590,020	167,221	37,944	6,310	501	155	264
	M:F	106.32		107.75	106.41	106.24	106.01	107.82	103.82	(110.4)	(105.8)	(102.6)
3	Male	863,818		647	79,059	427,821	276,231	68,898	10,204	614	152	192
	Female	814,013		611	74,313	404,120	259,152	65,523	9,413	595	112	174
	M:F	106.12		(105.9)	106.39	105.86	106.59	105.15	108.40	(103.2)	(135.7)	(110.3)
4	Male	610,618		40	12,449	182,991	291,133	107,499	15,404	804	166	132
	Female	576,718		52	11,757	173,129	275,001	101,015	14,716	767	145	136
	M:F	105.88			105.89	105.70	105.87	106.42	104.68	(104.8)	(114.5)	(97.1)
5	Male	428,039		2	2,210	56,899	209,027	135,324	23,090	1,186	190	111
	Female	405,095		6	2,009	53,821	197,199	128,856	21,885	1,074	144	101
	M:F	105.66			110.00	105.72	106.00	105.02	105.51	110.43	(131.9)	(109.9)
6	Male	302,050			567	14,841	115,205	137,645	31,923	1,601	217	51
	Female	285,034			567	14,099	108,867	129,806	30,006	1,419	212	58
	M:F	105.97			(100.0)	105.26	105.82	106.04	106.39	112.83	(102.4)	
7	Male	206,162			167	3,660	50,618	111,224	38,123	2,056	261	53
	Female	195,667			184	3,360	48,255	105,588	36,183	1,816	238	43
	M:F	105.36			(90.8)	108.93	104.90	105.34	105.36	113.22	(109.7)	
8	Male	134,130			64	877	18,273	73,788	38,463	2,364	262	39
	Female	126,950			53	822	17,502	69,928	36,170	2,228	219	28
	M:F	105.66				(106.7)	104.41	105.52	106.34	106.10	(119.6)	

TABLE 5 (CONTINUED)

9	Male	79,208			8	246	5,871	39,235	31,201	2,423	206	18
	Female	75,355			6	182	5,442	37,508	29,728	2,285	191	13
	M:F	105.11				(135.2)	107.88	104.60	104.95	106.04	(107.9)	
10	Male	42,314			2	66	1,642	17,412	20,972	2,047	161	12
	Female	39,683				67	1,547	16,298	19,697	1,917	145	12
	M:F	106.63					106.14	106.84	106.47	106.78	(111.0)	
11	Male	19,478				3	454	6,414	11,071	1,435	96	5
	Female	18,492				7	404	6,065	10,676	1,256	80	4
	M:F	105.33					(112.4)	105.75	103.70	114.25		
12 & over	Male	12,848					176	3,125	7,994	1,439	109	5
	Female	11,898				6	142	2,903	7,394	1,349	103	1
	M:F	107.98					(123.9)	107.65	108.11	106.67	(105.8)	
Order unknown	Male	1,943		38	467	636	372	249	86	7	3	85
	Female	1,881		32	429	584	447	224	84	10	—	71
Total	Male	5,613,578	174	145,224	1,420,429	1,779,263	1,240,515	767,302	239,999	17,064	2,180	1,428
	Female	5,287,821	171	135,862	1,334,819	1,675,964	1,169,382	725,783	226,855	15,758	1,912	1,315
	M:F	106.160	101.75	106.890	106.413	106.163	106.082	105.720	105.794	108.287	114.016	108.527

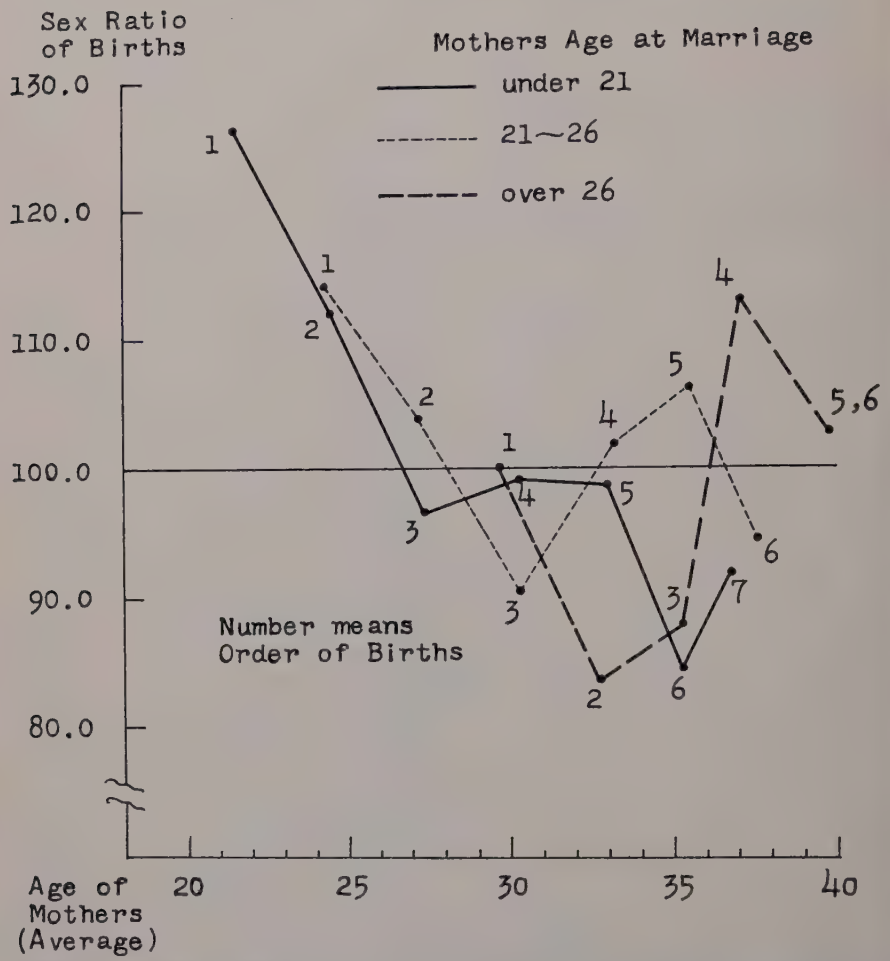

FIGURE 4. Sex ratio of different birth order in three groups divided by mother's age at marriage (after H. Komatsu).

to the mothers' age and, therefore, balanced the sex ratio at birth. But his material on 102,710 births is not enough to draw reliable conclusions when the number is divided into mothers' age classes.

Following Prinzing, Methorst[9] observed about 1.3 million live births, the sex ratio of the total being 105.1, and he found the sex ratio at birth is the largest in parents' age class 20–24. For the same age class of fathers the sex ratio is 108.2, and for the same age class of mothers it is 106.6. He thinks this is caused by the fact that mothers have more stillbirths the higher their age.

Both Bonnier and Methorst emphasize the fact that stillbirths increase as the age of the mothers increases. Although it is difficult to get the real number of stillbirths, either from those reported or by other methods, it seems our

data cannot be very different from the real conditions in Japan. Our results show the stillbirths ratio is highest in the age classes of mothers 15–19 and 40–49. In the mothers' age classes of 20–39 and over 50 the stillbirth rate becomes rather small.

On the effect of the fathers' age on the sex ratio at birth, we have few data in

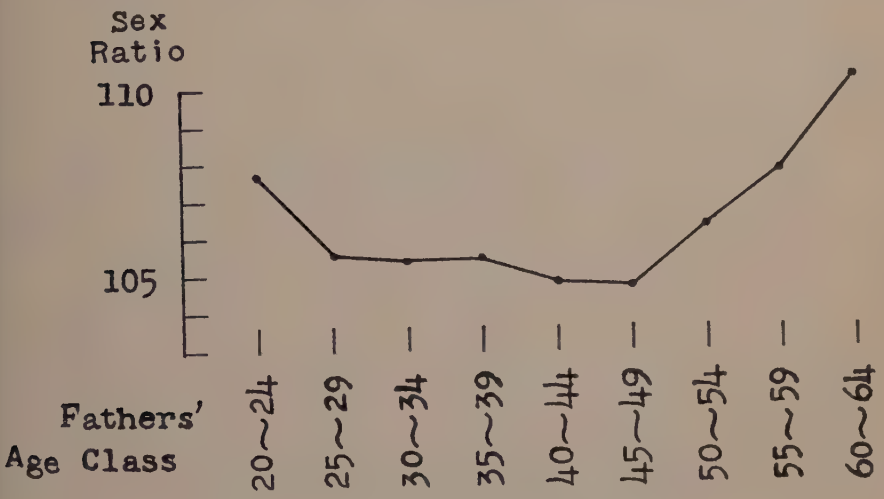

FIGURE 5. Sex ratio of births by fathers' age class in 1942.

TABLE 6

SEX RATIO OF 20,443 OFFSPRING OF 53 STALLIONS BY AGE GROUP SERIES

Age group of stallion	Sex of the offspring		Sex ratio of the offspring
	Male	Female	
5– 9 years	3,122	3,091	101.00
10–14	3,585	3,736	95.96
15–19	2,640	2,713	97.31
20–25	716	840	89.05
Total	10,063	10,380	96.96

(Data from Japanese National Stallion Pasture)

TABLE 7

DATA FROM BIDDER (SEX RATIO OF BIRTHS BY PRIMIPARA AND MULTIPARA)

	Age class of mother			Total
	Under 19	20–29	30 and over	
By primipara	111.7 ± 7.5	110.9 ± 12.2	116.9 ± 12.2	111.5 ± 3.4
By multipara	122.2 ± 27.5	108.2 ± 3.2	119.0 ± 4.4	112.4 ± 2.6

Number after ± means Standard error (SE).

SE = S $\sqrt{N/PQ}$, S: Sex ratio, P: Number of boys, Q: Number of girls, N = P + Q.

TABLE 8

DATA FROM COPEMAN AND PARSONS (SEX RATIO OF BIRTHS OF MICE)

Age of female at conception	Number of litters	Number of young			Sex ratio of births
		Total	Males	Females	
2 months	21	108	55	53	103.7
3–5	27	173	85	88	96.5
6	21	134	74	60	123.3

($\chi^2 = 1.1098$, $n = 2$, $0.7 > P > 0.5$).

TABLE 9

DATA FROM KING AND STOTSENBURG (SEX RATIO OF BIRTHS OF ALBINO RATS)

Litter series	Number of litters	Number of young			Sex ratio of births
		Total	Males	Females	
I	21	131	72	59	112.0
II	21	162	85	77	110.4
III	18	127	64	63	101.6
IV	15	96	41	55	74.5
Total	75	516	262	254	103.1

($\chi^2 = 3.6606$, $n = 3$, $0.5 > P > 0.3$).

TABLE 10

DATA FROM BIDDER (SEX RATIO OF BIRTHS BY MOTHERS' AGE)

Mother's age class	Number of births			Sex ratio of births
	Total	Boys	Girls	
14–19 years	967	512	455	112.5
20–29	7,644	3,992	3,652	109.3
30–34	3,032	1,641	1,391	118.0
40–	228	129	99	130.3
Total	11,871	6,274	5,597	112.1

($\chi^2 = 4.4273$, $n = 3$, $0.3 > P > 0.2$).

Japanese birth statistics. The curve of the sex ratio by fathers' age class in FIGURE 5 is similar to that of mothers' age classes. It seems variations of the ratio by fathers' age class are smaller than by mothers', except in the 20–24 class, which is similar to Methorst's data.

We cannot find a rise of sex ratio of births of older age in data on stallions; there is rather a tendency of the sex ratio to diminish according to increase of age. But C. Düsing[10] reported that the studhorses which covered more frequently had higher sex ratios at birth (FIGURE 6). This is another matter from that which we are now considering, but as a biological tendency we may refer to it.

We cannot be sure, from our observations, whether father or mother causes variation of sex ratio at birth according to age, or whether the mutual relation of parents' age has an effect, as is Hofacker-Sadler's theory, upon the sex ratio

at birth. But if the mothers' age should have an effect on the sex ratio of births, it might be considered that one of the most powerful reasons is as follows: Notwithstanding that what determines the sex of humankind and of mammals is not ova but spermatozoa, the secretions of the female sexual organs should be considered to have a selective effect upon *X*-spermatozoa and *Y*-(or *O*-) spermatozoa. Some mothers' constitutions might have an unfavorable reaction upon *X*-spermatozoa when the mother is very young and immature or is older and past normal maturity. This means an environment unfavorable for conception and, like the effects of a great war, a famine, abnormally hot temperature, *etc.*, should not be ignored. In such cases we might certainly think about the mother's age, not only her calendar age, but also with respect to the function of sexual organs—her biological age.

But such a relation, also, might not be inconsiderable upon the father's side.

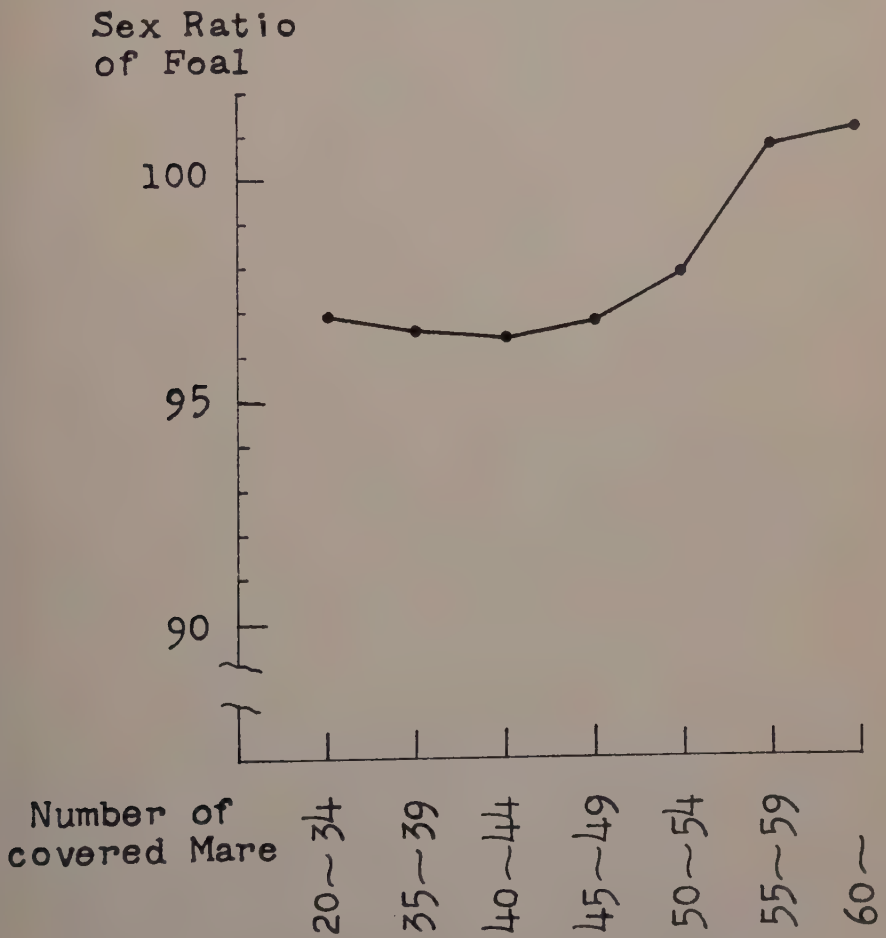

Figure 6. Sex ratio of foals classified by the number of covered mares (after C. Düsing).

Summary

From the Japanese vital statistics, we were able to conclude the following: The sex ratio of births has a variation according to mothers' age. When the mothers are under 20 the sex ratio of births is higher and then the ratio decreases gradually until the mothers are about 40–49. The sex ratio of births by aged mothers (over 50) is the highest. It has not yet been decided whether the last matter is a biological fact or the result of social influence.

There seems to be nearly the same tendency when births are divided by fathers' age classes.

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THE PROBLEM OF MONGOLISM*

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Introduction

Mongolism is the outstanding condition in the human being in which advanced parental age (specifically that of the mother) can be shown to be associated with an abnormal characteristic in the offspring.[1, 2] Age of itself is hardly to be considered among the direct causes of disease but is rather an index of intrinsic anatomical and physiological factors which directly influence the constitution of both individuals and groups.[3] The challenge in mongolism is to interpret the meaning of the data at hand; to formulate new avenues of approach; and, ultimately, to ascertain causation in terms of forces that can be controlled. It need scarcely be added that age—the mere accumulation of years—is not among the natural forces that mankind can control. The having of children under medical supervision at an advanced reproductive age is another matter.

The interests of preventive medicine and epidemiology have been greatly served in recent years by Gordon's interpretation of causation of mass disease as resident in three primary forces—those exerted by injurious agents, susceptible hosts, and variable environments—interacting in time.[4] Time assumes unusual importance in the problem of mongolism, for three periods are at issue: the age, that is to say the stage of development, of the embryo at the onset of the condition; the age of the mother when conceiving the defective embryo; and the month and the year when all determinents interact to cause the multiple defects which only become evident at birth. These considerations are all involved in the single question, When does mongolism occur?

Concern with mongolism has been so largely a preoccupation with age that it is well to emphasize that other simple and basic epidemiologic questions have been allowed to go by default; namely, where does mongolism occur, when, and at what rates? Not until the answers to these queries are known, in part at least, can medical investigation logically proceed to answer the questions of how and why this form of mental retardation happens, and what measures may be advanced in the interests of prevention.

When Does Mongolism Originate in the Fetus?

Several investigators working independently with specific kinds of clinical and pathologic material have reached approximately the same conclusion in respect to time of onset of mongolism in the embryo. Thus, Greig[5] in a detailed craniometric study of three mongoloid skulls found one of the most striking features to be the want of development of the alveolar processes of the upper jaw. He also observed that both nasal bones were absent in one skull, unilaterally absent in another, and relatively normal, although narrow, in the third (FIGURE 1.) Since ossification of the premaxilla is evident about the

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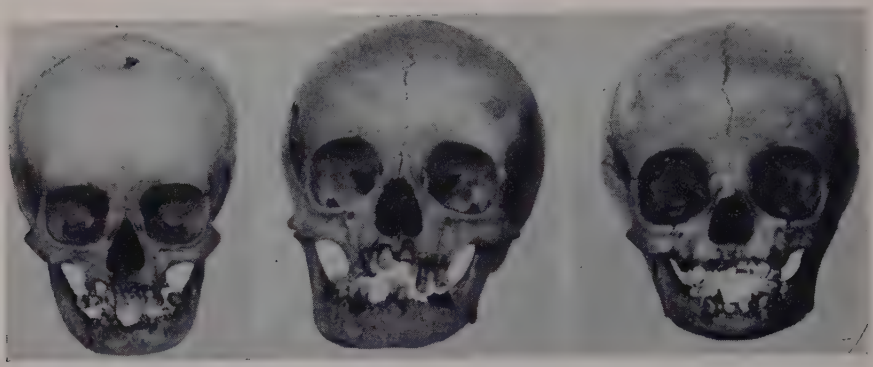

FIGURE 1. Three mongoloid skulls from girls 16, 14, and five years of age. From left to right they show bilaterally absent, relatively normal, and unilaterally absent nasal bones (Source: Greig[5]).

forty-second day of intrauterine life, and ossification of the nasal bones about the fifty-seventh day, Greig inferred that it is "during this period that the departure from normal growth shows itself, and this failure in the bones is accompanied or followed by defective growth elsewhere, notably of the nervous system." In the two skulls with unilateral or bilateral absence of the nasal bones, Greig observed that an upper lateral incisor tooth was also congenitally missing, once on the right and once on the left. Subsequently, Butler and I have confirmed the presence of such dental defects in a significant proportion of 47 mongoloid children studied at the Wrentham State School.[6] Twenty-three, about 45 per cent, were found to have dental abnormalities, generally localized to the permanent maxillary incisor teeth. The lateral incisor tooth was affected in 25 per cent of the patients with the gradient of deformity varying from absence of the tooth through various degrees of stunting.[6] The finding is interpreted as evidence of injury of the dental primordium, late in the second or early in the third month of pregnancy.

Cummings[7] studied dermatoglyphic features in the palm prints of 60 mongoloid children and found, among other characteristics, a transverse alignment of skin ridges in the distal region of the palm. He interpreted this anomaly to indicate that distinctive features of mongolism exist as early as the third and

TABLE 1

MONGOLISM AND MATERNAL AGE RATE PER 1000 MATERNITIES

	Maternal age in years							
	14–	20–	25–	30–	35–	40–	45–	Total
Incidence 1000 confinements	0.00	0.28	0.29	1.72	3.52	14.18	26.32	1.51
Number of confinements	2,902	17,826	20,530	14,526	8,244	2,186	152	66,366
Number of mongoloid births	0	5	6	25	29	31	4	100

Source: Carter & MacCarthy.[18]

FIGURE 2. X-rays of mongoloid hands showing absence and stunting of the middle phalanx of the fifth finger.

fourth fetal months, "the period in which the dermatoglyphics are differentiated in definitive form." Lowe[8] performed ophthalmoscopic examinations on 67 mongoloid individuals, largely adults, and found characteristic opacities situated in the fetal nucleus of the lens, speckling and peripheral atrophy of the iris stroma. He inferred that ocular development had progressed uneventfully during the early organogenic period of fetal life, following which a disturbance of differentiation had occurred at a stage of development corresponding to a fetal length of 35 mm (8.5 weeks of age). In addition, mongoloid patients generally have patencies of the cardiac interventricular septum and defects of the middle phalanx of the fifth finger (FIGURE 2).[9, 10] The significance of these collected findings cannot be calculated statistically, but it is definite and cumulative. The facts mutually support each other and an interpretation of a synchronous derivation of mongoloid anomalies somewhere between the sixth to ninth week of fetal life. This is the period when the nasal bones, the alveolar process, the dental lamina, the iris, the middle phalanx of the little finger and the cardiac septum are undergoing active differentiation.[11]

The search for causation is brought to focus on events of the first trimester of a pregnancy resulting in the birth of a mongoloid baby. The embryonic organism appears to have barely survived a critical period of intrauterine stress, the more tender buds being damaged, some never to regain their normal form of function while pre-existing and successional tissues escape injury. The multiple sites of injury indicate clearly that the disease is generalized in its active stage, and the universal presence of vascular anomalies implies that the morbid process is mediated through the vascular system. The inference has been drawn that mongolism is acquired after embryogenesis is all but complete, owing to adverse environmental factors.[12, 13, 14] Since the maternal or-

ganism constitutes the embryonic environment, the steps necessary to test the validity of these conclusions are epidemiologic inquiries to determine when, where, and how the maternal organism is so profoundly disturbed as to blight development of its own progeny.

When Do Mothers Produce Mongoloid Babies?

If mongolism has its onset in the fetus somewhere around the eighth week of gestation, a logical measure in determining causation of the mass disease is to plot its secular occurrence. No such data have ever been published, although Brushfield[15] has recorded admissions to a mental hospital in England of 54 mongoloid patients during the four years of World War I; 33 were admitted with the condition during the preceding four years, and 11 in the four postwar years. Such an increase of institutionalized patients during a war compared with a decrease thereafter carries a stronger implication of social upheaval and of custodial commitment than of a true rise in incidence. The possibility that mongolism actually did increase during "the war years when the ill health and worry were prolonged," or following 1918 pandemic influenza, is not to be discounted, however. Annual and seasonal rates of occurrence should be a matter of record and determined by date of birth, not by hospital admission.

Fragmentary studies of the occurrence of mongolism in Massachusetts from 1944 through 1945[13] suggest that the condition generally will be found to occur sporadically rather than in outbreaks. Such a supposition should not be completely taken for granted as is indicated by the epidemic occurrence of other anomalies—congenital cataract, heart defects, and deafness—in relation to rubella occurring in Australia during the early years of World War II. It may well be that the occurrence of mongolism, like cataract, in the progeny of younger women is more frequently related to infections in the second month of pregnancy than in the progeny of older women; and that near the menopause the condition has a significantly different etiology. The small group of women 20 years old and younger who produce mongoloid babies are certainly subject to different constitutional, gynecologic, operative, and social stresses than the small group of mothers aged 46 years and over.

For at least 25 years it has been clear that older women are more prone to bear mongoloid children than are younger women.[1, 2, 9] Indeed this was the first epidemiologic constant to be ascertained about mongolism, the first answer in what might be called an epidemiologic game of twenty questions designed to reveal causes. The fact has been proved over and over again, illuminating refinements being the demonstration that neither the father's age[16] nor the mother's parity[17] are determinants of the anomaly. Bleyer's data[2] demonstrates the distribution of mothers' ages in nearly 3000 collected cases compared with ages of mothers in the general American population. Although this demonstrates clearly the association of mongolism with the mothers' advancing years, it does not quantitate the risks of having a mongoloid child. These risks, which generally rise with mothers' age, have been calculated by Carter and MacCarthy[18] for a segment of the British population. In the interest of determining causation it is just as important to interpret the fact that young

mothers may rarely produce a mongoloid baby, as it is to interpret the more dramatic fact that mongolism stands in proven relation to the reproductive performance of older women.

Where Does Mongolism Occur?

Unfortunately, congenital anomalies are not reportable conditions and this fact poses a critical obstacle to mass definition of mongolism by place of mother's residence in early pregnancy. Yet geographical orientation of any morbid process is one of the primer objectives of epidemiologic study, as elementary and necessary for mass analysis as is the determination of pulse, temperature, or respiratory rate for clinical diagnosis of the sick individual. Gordon has defined the principle as follows:

"Disease is oriented into the region or district in which it occurs through collecting all information bearing on prevalence . . . and the origin of epidemics. The material is then organized according to a coherent scheme. The region is first described in terms of . . . ancient and current factors of historical geography, the customs and usages of cities and villages . . .; attention then turns to statistical features of occupation, wealth, political constitution and the vital phenomena of births and deaths. The special physical description of the locality receives somewhat more weight, with attention to the soil, agriculture products, water, climate and weather."[19] Attempts at such an approach to mongolism are rudimentary, to say the least, although one is of sufficient scope to be mentioned here.

Myers,[20] took into account maternal residence in analyzing factors antedating birth in a series of 215 mongoloid children and 215 non-mongoloid, mentally defective controls. Finding a 9 to 1 greater frequency of "recognized thyroid disorders," including both hypo- and hyperthyroidism among mothers of mongoloid babies, he proceeded to test the hypothesis that a geographical distribution of afflicted babies might show a predilection for "those areas in which thyroid disorders are more frequent." This appeared to be the case as shown in the map (FIGURE 3), prepared by dividing 64 geographical units of Ontario into four main sections on the basis of what Myers called "thyroid rates"—that is to say, ratios of reported deaths from diseases of the thyroid gland to deaths from all causes. Artifact may well have intruded into the results, but a check of Myers' hypothesis would come from a study of the incidence of new cases of mongolism per 1000 live births in each of the four areas with different "thyroid rates" provided that the same care was used in case finding in each of the four areas. The circumstance that it is not clear whether maternal hypothyroidism, hyperthyroidism, or both are suspect of being members of the causative complex is less important than the task of confirming the basic abnormality in distribution of mongolism suggested by Myers' data. Both hypothyroidism and hyperthyroidism, occasionally found associated with mongolism in clinical experience,[21, 22] have been advanced to explain the syndrome. Such impressions remain speculative until tested in groups of people.

A main obstacle to the reporting of mongolism is the difficulty in making the diagnosis at birth. This can be done, however,[23] and confirmed at a later

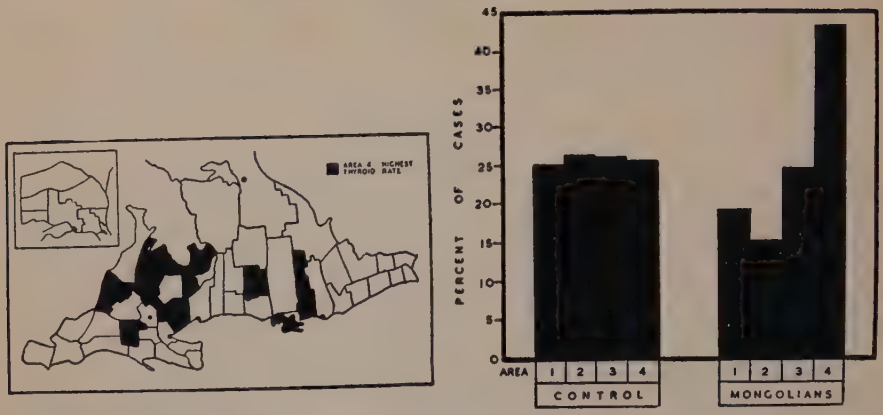

FIGURE 3. Left: geographical areas (area 4) of the Province of Ontario with highest "thyroid rates." Right: distribution of mongoloid and nonmongoloid patients into four birth areas of Ontario (Source: Myers[20]).

time. Undoubtedly in the past many, if not most, babies with mongolism have been discharged from lying-in hospitals unrecorded on the records, as the mother left the obstetrical field for the province of the pediatrician—time again playing a significant role not only in the pathogenesis of the disease, but in its recognition.

How Mongolism Originates

A mature interpretation of the etiology and pathogenesis of mongolism awaits the accumulation of further data. A constitutional factor of the mother that is worth investigating further is the significance of retroflexion of the uterus—observed by Schröder about five times more frequently in the mothers of mongoloid babies than in mothers* of normal children. I also have been impressed by the proportion of mothers of affected babies who were aware of having this condition, and wonder if it may not predispose to abnormal placentation and hemorrhage. Elsewhere[13] I have emphasized that between 20 and 25 per cent of the more than 200 mothers studied by Beidleman,[24] Benda,[10, 14] and Schröder[25] were found to have had significant vaginal bleeding—presumably representing circulatory embarrassment and a critical stress for the fetus. Lack of oxygen to the fetus may operate in such "threatened abortions," and experiments undertaken at the Harvard School of Public Health have shown that temporary anoxia may indeed be teratogenic for the rodent embryo.[27]

The mongoloid child is thought to have weathered a period of critical, almost lethal stress as an embryo and to have survived, but with permanent arrest of tissues differentiating during the period of stress. The hypothesis that mongolism is a stage specific defect of about the eighth week of fetal life, induced in a potentially normal embryo by a concatenation of acute or chronic events[26] is not yet wholly proven. However, the import of mass studies of the past quarter of a century is in this direction. It should be possible to confirm the correctness of this conclusion and even begin a program of control

* Not matched, however, by age and parity.

measures by the end of the present decade if as much weight is given to epidemiologic considerations as is accorded to clinical diagnosis and custodial care of these blighted beings. Whatever the important metabolic, anatomical, or infectious agents turn out to be, it seems reasonably clear that they will number, not one but several. With an increased understanding of these multiple causative factors, much can be done in future years to limit the occurrence of the syndrome.

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THE INFLUENCE OF MATERNAL AGE ON DEVELOPMENT OF THE SKELETON OF THE MOUSE

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For centuries, anatomists have been aware of the existence of variation in the bones, muscles, blood-vessels, *etc.*, of the human body. Skeletal variation, being the most tangible, has been much studied and in 1912 Le Double published an exhaustive treatise on variation in the human vertebral column, dealing in detail with individual vertebrae. He could not go deeply into the causes of the anomalies but he was able to show that the frequencies of some variants differ in different races and that similar variants occur in other mammals, especially Primates.

Only recently has similar polymorphism been found in the mouse. Of course, skeletal defects associated with specific mutant genes, such as grey lethal, brachyury, luxate, and so on, have been known for some time. Differences between and within inbred strains in the position of the thoraco-lumbar and lumbo-sacral borders have also been studied, Green (1941) showing that in the Bagg albino strain about 8.5 per cent of the total variance at the lumbo-sacral border is connected with age of mother. But only in 1950, with Grüneberg's paper on skeletal variation in the vertebrae of mice from various pure lines, and Weber's on similar variation in wild mice, was it realized that the mouse skeleton, like man's, is extensively polymorphic and that pure lines seem to be especially variable in this respect.

This variability, like that described by Le Double, extends throughout the vertebral column but is greatest in the cervical region; the skull and appendicular skeleton are also affected. Strains A/Gr and C57BL/Gr, studied by Grüneberg and analyzed here, show examples of anomalous fusions and failures of normal fusion, incomplete transverse foramina, shifts in the positions of vertebrae or parts of vertebrae and so on. Structures normally present, such as the tuberculum anterius on the sixth cervical, may be absent. Some anomalies are confined to one pure line, some are rare in one but common in the other.

The manifestation of these variants must depend partly on the genetic background, for their frequency differs in different pure lines. Even within the pure lines studied, sex and subline differences are often large. But, allowing for these differences, variation is still extensive within highly inbred strains, which shows the overriding importance of nongenetic factors. So this is excellent material for a study of how the environment mediates between gene and character. It should give a more balanced picture than hitherto of the importance of maternal age as a cause of skeletal variation. And since the bone anomalies are often accompanied by changes in muscles, blood-vessels and so on (more difficult to study and therefore less well known), the causes of variation in other tissues are probably similar to those in bone.

The skeletal material used in this analysis was prepared by the papain process of enzymatic maceration, which cleans and disarticulates each bone. Most of

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the 472 A/Gr and 735 C57BL/Gr preparations were the offspring of paired matings set up in October 1949. The members of each pair were littermates, so possible effects of paternal age cannot be separated from those due to age of mother. Correlations between maternal age and parity are 0.73 for strain A and 0.82 for C57BL, so it is not always certain which of these two factors is at work.

Most of the total variance, over 80 per cent in three quarters of the characters, is due to intangible nongenetic factors acting independently on individuals or sides of individuals. But significant trends with maternal age and/or parity occur in seven out of twenty-one anomalies sufficiently common in the two strains for full analysis, accounting on the average for about 10 per cent of the total variance with respect to these seven.

One pronounced trend is with a relatively rare character in the C57BL strain, namely cranial dystopia of the processus spinosus of the second thoracic vertebra (illustrated by Grüneberg, 1950). The only vertebra normally carrying a well-developed spinous process is Th II. This process, which is the origin for a number of neck muscles, is generally spatulate; when shifted on to Th I it is nearly always in the form of a long rod. The frequency of this dystopic spinous process (TABLE 1) sinks to zero in the offspring of old mothers, accounting for about 35 per cent of the total variance. With birth order there is a similar but less extreme trend.

The Th II p.s. varies greatly in size even in the absence of any shift on to Th I. The proportion well developed increases with maternal age. This trend, accounting for 4 per cent of the variance, is almost certainly due to maternal age alone, for birth order has little effect when mice within a limited maternal age range are tested.

There is a tetrachoric correlation of 0.30 ± 0.13 between cranial dystopia of the p.s. of Th II and a cranial shift of the lumbo-sacral border (normally there are twenty-six presacral vertebrae). Because of this, and of Green's findings in Bagg albinos, it is not surprising that in C57BL (but apparently not in A) there is a significant downwards trend in the incidence of dystopic sacrum with increasing maternal age (TABLE 2), accounting for 9 per cent of the total variance. It seems fairly certain that maternal age is the operative factor here, as the trend with birth order does not approach significance. TABLE 2 is based only on one of the two C57BL substrains used, as there has been considerable genetic divergence between the two, sacral dystopia being uncommon in the

TABLE 1

MATERNAL AGE AND CRANIAL DYSTOPIA OF THE P.S. OF TH II IN C57BL

Age of mother	P.s. dystopic	P.s. normal	Total	% dystopic
Below 131 days	6	90	96	6.3
131–160	6	115	121	5.0
161–190	4	84	88	4.5
191–220	1	90	91	1.1
221–250	0	68	68	0.0
Above 250	0	103	103	0.0

TABLE 2
MATERNAL AGE AND DYSTOPIC SACRUM IN C57BL

Age of mother	Affected	Unaffected	Total	% affected	χ^2
Below 131 days	17	32	49	34.7	9.0
131–160	16	54	70	22.9	1.1
161–190	7	55	62	11.3	1.9
191–220	12	50	62	19.4	0.0
221–250	5	33	38	13.2	0.6
Over 250	5	57	62	8.1	4.2

$\chi^2 = 16.8$, n = 5, and P = 0.005.

TABLE 3
MATERNAL AGE AND CAUDAL DYSTOPIA OF A STRAIN THORACIC ARCH FORAMINA; ONLY MICE WITH FORAMINA INCLUDED

Age of mother	Affected	Unaffected	Total	% affected
Below 161 days	14	114	128	10.9
Above 160 days	3	113	116	2.6

$\chi^2 = 5.33$, P = 0.02.

FIGURE 1. Fifth thoracic vertebrae of A strain mice, showing arch foramina (Camera lucida drawings).

other. There are sex differences in both the A and C57BL strains, as in Bagg albinos.

The fifth thoracic vertebra of A strain mice usually carries on the left side of its arch one or two foramina, by which blood-vessels pass from within the vertebral column to join a vein leading from the scapular portion of the "hibernating gland" into the azygous vein. These foramina, shown in FIGURE 1, may be shifted on to Th VI. TABLE 3 shows that this happens less frequently in the offspring of older mothers, a trend which accounts for about 11 per cent of the total variance. Here again the trend with parity is less marked and not significant. Owing to the rarity of these arch foramina in C57BL, no analysis was possible.

Grüneberg (1951) has shown that 17.9 per cent of CBA/Gr mice lack one or more third molar teeth, absence being associated with the generally small size of these molars in the strain and their great variability. A similar defect, but of lower third molars only, occurs in 2.3 per cent of A mice. FIGURE 2 shows that these teeth are, on the whole, smaller and simpler in A than in C57BL, where only one out of 735 mice lacked a lower third molar. This mouse had other unique skeletal abnormalities, suggesting that a different mechanism from that in A is responsible for loss of the tooth. There is a striking tendency for this anomaly to occur in A first litters, the probability of obtaining by

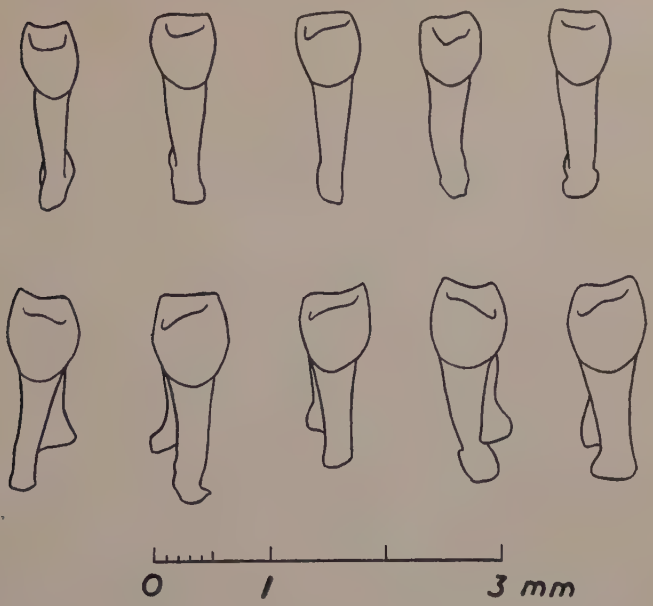

FIGURE 2. Lower third molars of strains A (above) and C57BL (below) (Camera lucida drawings).

TABLE 4
PARITY AND ABSENCE OF A LOWER THIRD MOLARS

Litter	Affected	Unaffected	Total	% affected
First	7	104	111	6.3
Later	1*	203	204	0.5

* In a second litter.

TABLE 5
MATERNAL AGE AND ABSENCE OF A LOWER THIRD MOLARS

Age of mother	Affected	Unaffected	Total	% affected
Below 131 days	6	90	96	6.3
131–160	2	61	63	3.2
Over 160	0	156	156	0.0

chance the distribution shown in TABLE 4, or a more extreme one, being 0.0033, as calculated by Fisher's exact method.

Maternal age also has a striking effect (TABLE 5); it will be necessary to obtain many early litters from old mothers in order to decide which of these factors is the important one. The trend shown here has been confirmed in later experiments, the only four affected mice being from the first litters of mothers less than one hundred days old. In CBA, this anomaly is probably not connected with maternal age or parity (Grüneberg, 1951). Twice in the A strain two littermates were affected, suggesting that here as in CBA there

is within-litter bunching of abnormals, the result of maternal influences acting on litters as a whole.

Grüneberg found that the smaller the potential size of a CBA third molar, the more likely it was to be absent. The same situation exists in the A strain. Thus the concentration of this anomaly in first litters is associated with smaller size; the mean bucco-lingual diameter of these teeth is 0.531 mm in first litters and 0.548 mm in later litters. The difference—0.017 ± 0.0047 mm—is significant, assuming that the correction for heterogeneity in the data due to bunching is small.

There is a partial correlation of about 0.25 between lower third molar size and birth-weight, eliminating weight at four weeks; the correlation is nearly 0.6 between tooth-size and weight at four weeks, eliminating birth-weight. Birth-weights of mice in first litters of seven are significantly less than those in later litters of the same size ($t = 2.39$, $n = 94$, and $P = 0.02$); with litters of five or six, the trend is similar but not quite significant ($P = 0.06$). Similarly, at four weeks, mice in first litters weigh on the average about two grams less than those in later litters. Thus it appears that in the A strain, under the conditions of this experiment, pre- and postnatal growth of mice in first litters of young mothers was retarded when compared with later litters.

Dietary and seasonal influences may well alter this state of affairs; here the diet was suboptimal and the litters were born in the winter and early spring. Litters from the same subline born the following summer had larger third molars, none of which were absent. On the suboptimal diet, litter-size tends to be largest in offspring of young mothers (TABLE 6); since mice in large litters weigh less at birth, this trend also helps to account for the smaller size of lower third molars in first litters and the absence of teeth as a result. The trend with parity is similar, but not quite so marked. On a better diet, giving larger litters, this trend with maternal age does not occur.

All these results show a decline in the frequency of the abnormal skeletal variant as maternal age increases. This is also true for certain other anomalies not described here, in which the effect is less, and, in general, for pure line variants studied by other workers. Thus Wright (1934) found that in his inbred guinea-pig strain the frequency of polydactyly decreased in the offspring of older mothers. Holt (1948) found the same trend in homozygous matings of a polydactylous strain of mice, and Green (1941) with variation at the lumbo-sacral border in Bagg albinos. With harelip, however, Reed (1936) found no straightforward trend; offspring from mothers in the three month age-group had a significantly lower percentage of harelips than those from mothers in the preceding or the following age-groups.

TABLE 6

MATERNAL AGE AND LITTER-SIZE IN THE A STRAIN ON A SUBOPTIMAL DIET

Age of mother	Number of litters	Total born	Mean litter-size
Below 101 days	15	102	6.80
101–160	31	153	4.94
Over 160	28	136	4.86

In outbred populations, trends with maternal age and/or parity usually seem to be in the opposite direction. Disturbances due to sensitization, such as *Rhesus* incompatibility, are more likely to occur in later pregnancies. The incidence of mongoloid idiocy rises with maternal age; similarly that of gross malformations of the central nervous system and central placenta previa (Penrose, 1939). These are all pathological abnormalities, subjected to forces of natural selection, which one might expect to be weaker near the upper age-limit of child-bearing. But of those characters in inbred strains which show a relation to maternal age only harelip is clearly pathological; it is this particular anomaly which is an apparent exception to the general trend in pure lines.

Effects of maternal age changes are mainly confined to the group of variants comprising cranial and caudal dystopias, as far as this investigation is concerned. Variation in the spinous process of Th II should probably be included in this group, since it seems to depend on the position of a dorsal mass of mesenchyme out of which the spine condenses. Of the variants in which there has been failure of a tube to close normally (dyssymphysis of the axis posterior arch, of the arch of Th I, of the ischium and pubis; open foramina transversaria) only with open foramina of the third to sixth cervical vertebrae is there significant heterogeneity. This is probably due to an effect of birth-order, first litters having more foramina open on the average. In none of the various types of fusion encountered is there a maternal age effect. But, with some types of fusion and dyssymphysis, litter-size or length of gestation is one of the causes of variation.

This investigation shows that within pure lines maternal age can account for between zero and 35 per cent of the total variance in different skeletal anomalies. In outbred stocks, which have more genetic and probably less environmental variance, the influence of maternal age may well be smaller. Maternal intrauterine changes with age seem, on the whole, to lead to some decrease of skeletal variation in the offspring, judging from the trends found. It seems likely that dietary factors may modify these trends and a nutritional approach might give useful information on their physiological basis.

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THE INFLUENCE OF AGE OF MOTHER ON PATTERN OF REPRODUCTION*

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In attempting to develop isogenic races of rabbits for experimental study of normal growth processes, it has become increasingly apparent that this species transmits an unusual number of lethal or sublethal genes which manifest themselves at various stages in the reproductive cycle. Six major characteristics, including fertility, fecundity, lactation, maternal behavior, growth, and viability, are recognized (Sawin and Curran, 1952). The greatest expression of each of these is apparently influenced by several subsidiary factors, failure of any one of which can be a serious handicap, and of more than one may be disastrous. For example, maximum fertility is dependent upon functional gonads, viability of gametes, and normal functioning of ducts and glands. Each, however, must be matched by an equivalent relative capacity of each of the other five. Imbalances, such as high fecundity (litter size) and impaired lactation capacity, can be as detrimental as complete failure of either one. Extraneous factors, such as the age of the mother acting differentially upon any one, more than on another, even though only to a minor degree, can be of major importance, both directly and indirectly.

The observations reported at this time are a part of results obtained in efforts to determine the fundamental nature of interracial variations in reproductive patterns occurring in ten different races being bred at this laboratory. They are restricted to two races, *X*, descended from the small-sized race of Castle (1929), and *IIIc* of New Zealand White extraction, being the two upon which most information is now available. They are still very incomplete.

The most significant effect of the age of the mother upon the young which we have observed thus far is that upon viability, which is significantly different in these two races. In the rabbit the three major causes of mortality during growth and development are coccidiosis, pneumonia, and enteritis. The pathology of the first two of these is understood and is traceable to specific organisms which are controllable. The third, manifested by mucous discharge from the rectum, diarrhea, bloat, and, on autopsy, generalized inflammation of the bowels, has no known specific causative agent. However, as is also the case with pneumonia, it is responding to improved methods of care and management, particularly those which provide better ventilation of quarters, elimination of drafts and dampness and better sanitation. In both of these the inherent resistance of the individual seems to be a major factor (Sawin and Curran, 1952). Both races have been maintained under as nearly the same environmental conditions as possible.

Previously it was found that the young of race *X* tend to die earlier than race *IIIc*, but more of them actually reach maturity. It has further been found that, as shown in TABLES 1 and 2, the mean life span or age at death is

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TABLE 1
AGE OF MOTHER AND LIFE SPAN OF YOUNG
(Race X (1950–51))

Age of mother in months	Life span of young in months						Total	Mean
	1	2	3	4	5	6+		
6	1	—	2	4	1	7	15	4.6 ± .40
7–12	40	16	17	9	9	59	150	3.7 ± .17
13–18	34	5	7	5	7	25	83	3.3 ± .24
19–24	6	4	3	2	2	5	22	3.2 ± .42
25–30	4	3	—	1	—	—	8	1.8 —
Total	85	28	29	21	19	96	278	3.5 —

TABLE 2
AGE OF MOTHER AND LIFE SPAN OF YOUNG
Race IIIc (1950–51)

Age of mother in months	Life span of young in months						Total	Mean
	1	2	3	4	5	6+		
6	21	—	8	3	—	2	34	2.02 ± .26
7–12	71	28	15	7	9	44	174	2.92 ± .16
13–18	24	13	6	4	8	20	75	3.25 ± .24
19–24	30	6	3	1	5	5	50	2.20 ± .26
25–30	8	2	2	2	3	5	22	3.23 ± .44
30+	7	—	—	—	—	—	7	— —
Total	161	49	34	17	25	76	365	2.8 —

a maximum in the offspring from young race *X* mothers, and decreases inversely with the age of the mothers, whereas in race *IIIc* the tendency is for the life span of the offspring to increase as the age of the mother increases up to 18 months, following which there is the same tendency as in race *X* for the viability of the young to decline.

It is of further interest to note that there is a significant racial difference in mortality during late prenatal development and at birth. In race *X* (FIGURE 1) there is a greater mortality at birth than prenatally at all ages of the mothers except in the early history (prior to 1947) when there was some tendency for the situation to be reversed in the early and late age groups. In contrast, race *IIIc* (FIGURE 2) manifests a greater incidence of prenatal mortality during the last stages of pregnancy, suggesting a significant difference in the physiological constitution of the mothers of the two races.

Since mortality can be influenced by events which happen some time previous to death, we have considered other maternal differences which conceivably could influence the young, and lactation capacity of the mother is a logical suspect. We already know (Sawin and Curran, 1952) that there is a significant difference in the lactation capacity of these two races. Race *IIIc* tends to be significantly inadequate in the 15–21 day period just prior to the age at which the young can utilize solid food to advantage. FIGURE 3 shows that when the

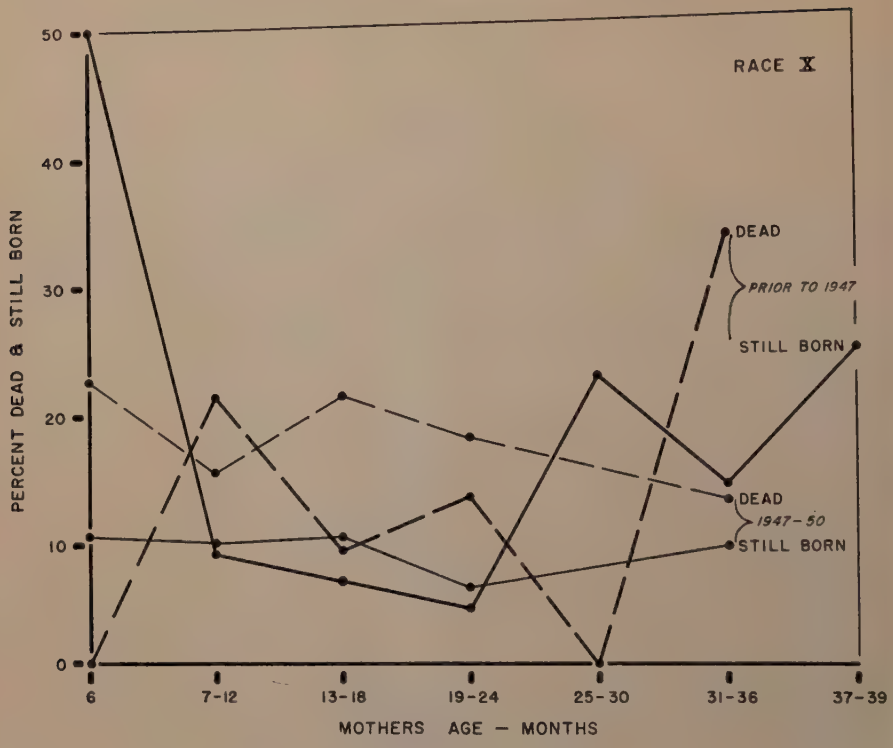

FIGURE 1.

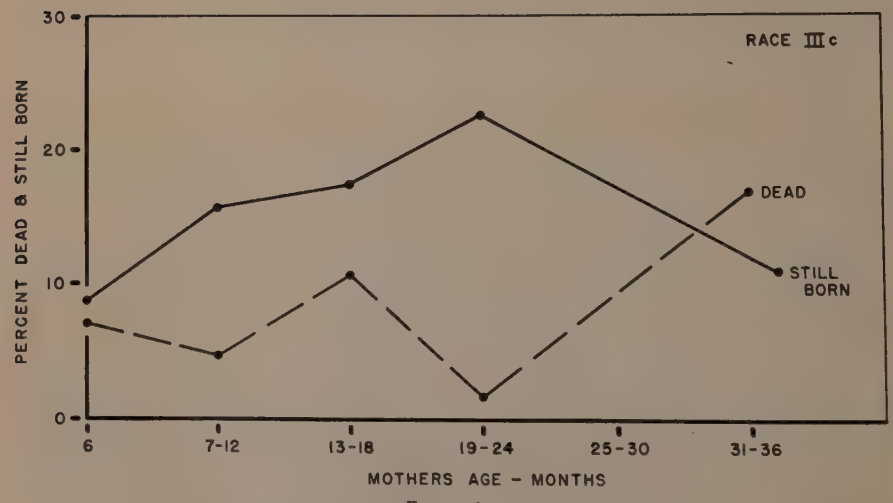

FIGURE 2.

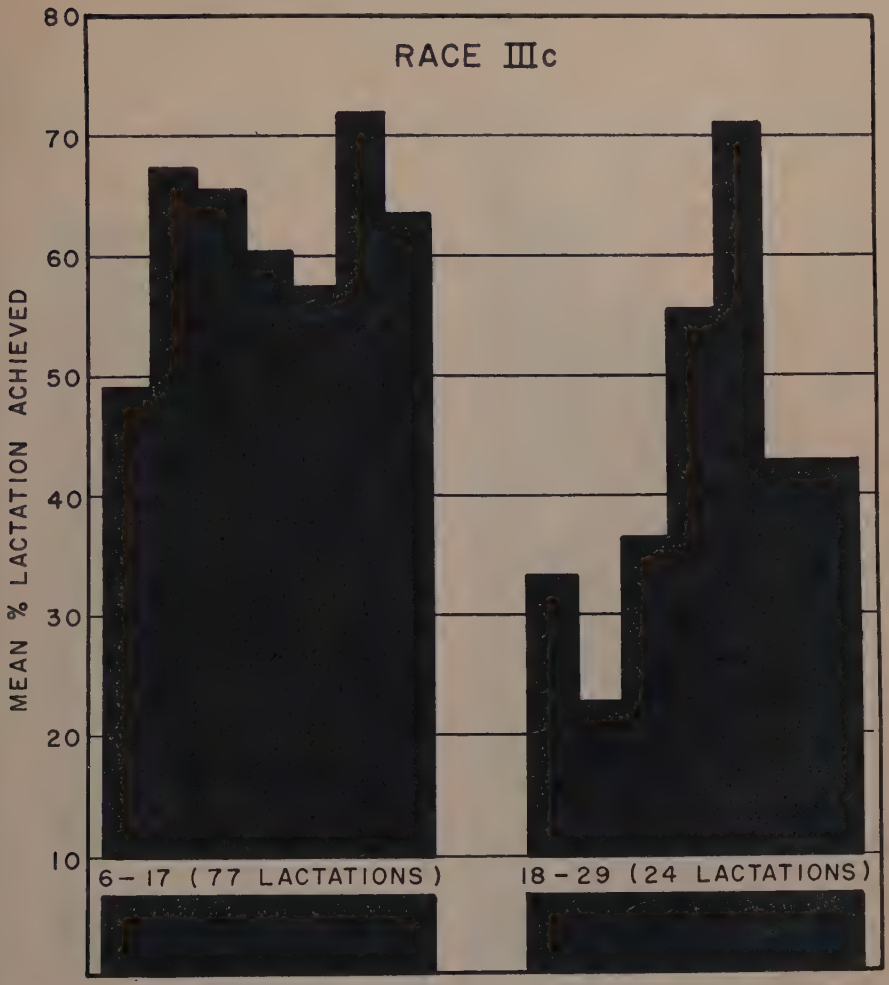

FIGURE 3.

milk capacity of the mother is estimated, at birth and during the first six days of lactation, on the basis of the per cent of young filled to capacity, the older mothers (over 18 months) of race *IIIc* are on the average slower in achieving full lactation as compared with those under 18 months, and they tend to decline by the fifth day. Such an age difference is not manifest in race *X* (FIGURE 4).

Since viability of the young is lowest in race *IIIc* mothers of the younger age groups when lactation is best, and in race *X* there is no difference between age groups, it seems unlikely that inadequate lactation is a major factor in the observed differences in viability with age of mothers.

Another factor which might influence the viability of the developing young

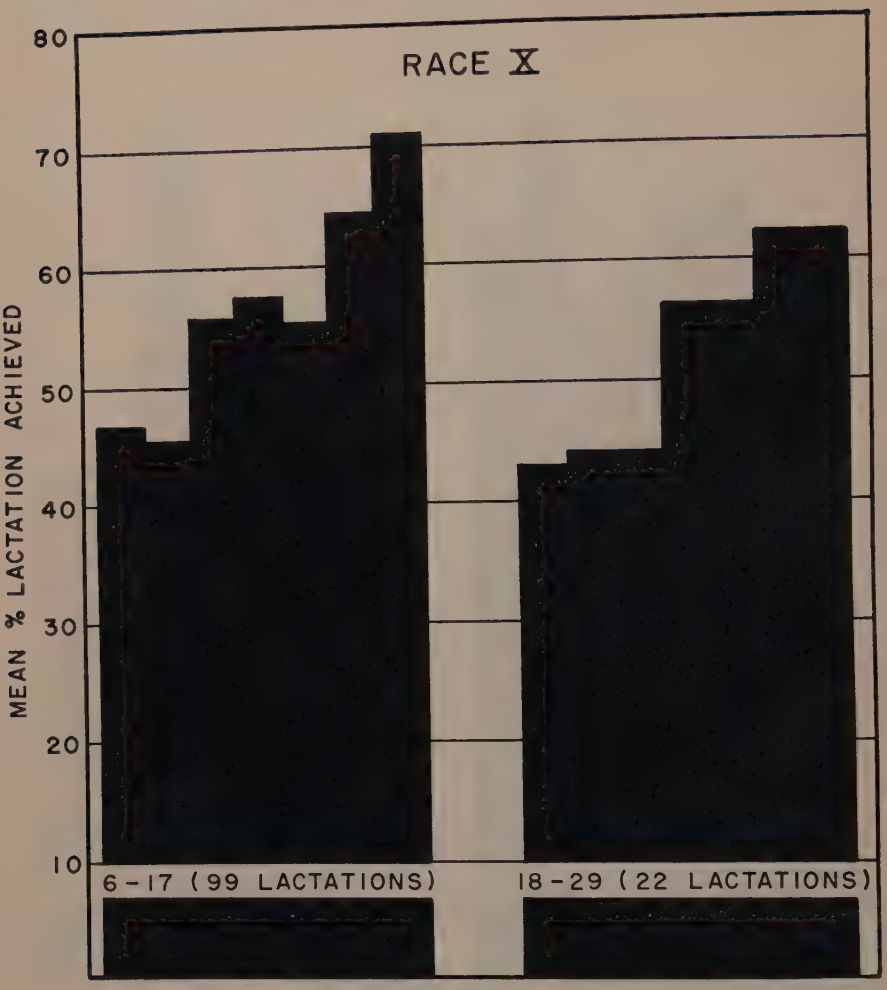

FIGURE 4.

is maternal behavior or care. TABLE 3 shows that there are significant differences between the mothers of the two races with respect to six of twelve characteristics of maternal care for which it is possible to rate them.

These are: (1) the location of the nest, whether in the nestbox provided or outside of it, which gives protection to the young; (2 and 3) the time of building and lining of the nest with hair plucked from the mother's body; (4) the amount of nest lining; and (8 and 9) aggressive protection of the young. Two kinds of aggression are apparently extant in these two races. Race *X* manifests a general type of aggression between individuals and toward attendants in both sexes which appears early in life as well as during maturity and lactation. Race *IIIc* mothers rarely manifest aggression other than as a maternal characteristic.

TABLE 3
RACIAL DIFFERENCES IN MATERNAL BEHAVIOR

Characteristic	Race		χ^2	P
	X	IIIc		
	Per cent of abnormality			
1) Nests built outside of box	5.9 (152)*	21.6 (171)	16.5	<.01
2) Nests built postpartum	36.2 (152)	64.6 (147)	23.7	<.01
3) Nests lined postpartum	43.4 (152)	69.3 (147)	19.4	<.01
4) Poor nests	46.4 (155)	37.2 (148)	2.3	.1–.2
5) Inadequate nest lining	69.2 (156)	34.4 (160)	23.3	<.01
6) Young cannibalized at birth	10.3 (165)	15.0 (180)	1.3	.2 – 3
7) Young scattered at birth	10.9 (165)	22.7 (180)	2.1	.05–.10
8) Mothers aggressive prepartum	56.2 (112)	15.2 (131)	43.2	<.01
9) Mothers aggressive postpartum	62.6 (91)	31.8 (88)	15.8	<.01
10) Nests fouled prepartum	23.5 (149)	32.4 (139)	2.4	.1–.2
11) Nests fouled postpartum	32.2 (93)	29.9 (87)	.03	.8–.9
12) Maternal solicitude	12.4 (97)	16.9 (83)	.18	.5–.7

Numbers in ( ) = Number of parturitions observed. Underscoring directs attention to the race with greater incidence. Double underscore indicates high statistical significance.

TABLES 4 and 5 show, however, that in only five of these characters is there any indication that the age of the mothers makes any difference. In three cases (TABLE 4) the nature of the distribution and inadequate numbers tends to minimize the significance at this time.

TABLE 5 shows that in race *X* the generalized aggression before parturition tends to increase with age, from 49.3 per cent in mothers 6–17 months of age to 84.2 per cent in mothers over 18 months. After parturition older mothers are not significantly more aggressive than younger mothers. Thus, it does not seem likely that this character could have any influence upon the change in viability of the young which has been noted. In race *IIIc* there is a reduction in maternal aggression in the older mothers which is significant at the 0.5 per cent level of probability, which, if anything, might denote a decline in maternal instincts. Since we lack significant evidence of such in any of the other characters, however, it seems doubtful if the differences in viability noted can be attributed to differences in maternal care.

An alternative possibility is that there is some more subtle physiological influence acting prenatally, or by way of the mother's milk. Direct evidence of such is not available at present, but other racial differences in the reproductive pattern of these mothers constitute indirect evidence of endocrine deficiencies or imbalance which may be suspect. Both races have an unusually high proportion (49.9 per cent in race *IIIc* and 45.8 per cent in race *X*) of unsuccessful matings but, as shown in TABLE 6, it is not influenced significantly by the age of the mother. As shown in FIGURE 5, both races have a good average litter size in proportion to adult body size (Castle and Gregory, 1929), and it is not unusual for some lines of *IIIc* to have litters of more than 10, which suggests an unusually high ovulation rate. This has been confirmed at

TABLE 4
INFLUENCE OF AGE UPON MATERNAL BEHAVIOR

Character	Race		Age mother in months 6–17	18–29	30+	Total parturitions	χ^2	P
1) Nest displacement	X	No. parturitions	79	20	2	101	.83	.5–.7*
		% displaced	3.8	.6	0.6			
	IIIc	No. parturitions	64	12	6	82	1.27	.5–.7
		% displaced	12.5	16.7	0.0			
2) Time of nest building	X	No. parturitions	78	20	2	100	.31	.8–.9
		% postpartum	35.9	40.0	50.0			
	IIIc	No. parturitions	62	11	6	79	9.48	<.01
		% postpartum	69.3	90.9	33.3			
3) Time of nest lining	X	No. parturitions	79	20	2	101	.68	.7–.8
		% postpartum	44.3	50.0	50.0			
	IIIc	No. parturitions	62	12	6	80	6.83	.02–.05
		% postpartum	79.0	83.3	33.3			
4) Nature of nest	X	No. parturitions	78	21	—	99	.11	d.f. = 1
		% poor	48.7	45.0	—			.90–.95
	IIIc	No. parturitions	63	11	6	80	5.75	.05–.10
		% poor	34.9	27.2	16.6			
5) Quantity of nest lining	X	No. parturitions	81	21	—	102	2.22	d.f. =1
		% poor	65.4	47.7	—			.1–.2
	IIIc	No. parturitions	68	12	6	86	1.01	.5–.7
		% poor	36.7	33.3	16.6			
6) Cannibalism	X	No. parturitions	89	20	1	110	1.46	.3–.5
		% cannibalized	14.6	5.0	—			
	IIIc	No. parturitions	77	15	6	98	3.38	.1–.2
		% cannibalized	10.4	26.7	—			
7) Scattering	X	No. parturitions	88	20	1	109	.798	.3–.5
		% scattered	17.0	10.0	—			
	IIIc	No. parturitions	74	15	6	95	1.06	.3
		% scattered	28.4	20.0	—			

* d.f. = 2 except where otherwise indicated.

TABLE 5
INFLUENCE OF AGE UPON AGGRESSION (MATERNAL AND NONMATERNAL)

Character	Race		Age mother in months 6–17	18–29	30+	Total parturitions	χ^2	P
Aggression before parturition	X	No. parturitions	73	19	—	92	7.53	<.01
		% aggressive	49.3	84.2	—			
	IIIc	No. parturitions	65	14	5	84	2.52	.1–.2
		% aggressive	15.4	28.6	None		d.f. = 2	
Aggression after parturition	X	No. parturitions	50	14	—	64	.61	.7–.8
		% aggressive	60.0	71.4	—			
	IIIc	No. parturitions	35	18	—	53	5.18	.02–.05
		% aggressive	48.5	16.7	—			

TABLE 6
PER CENT OF MATING FAILURES

	Age of mothers in months						Total matings
	5–6	7–12	13–18	19–24	25–30	31–38	
Race X	49.6	46.3	49.8	45.8	46.5	48.1	1108
Race IIIc	37.5	55.8	49.8	59.7	40.6	44.4	310

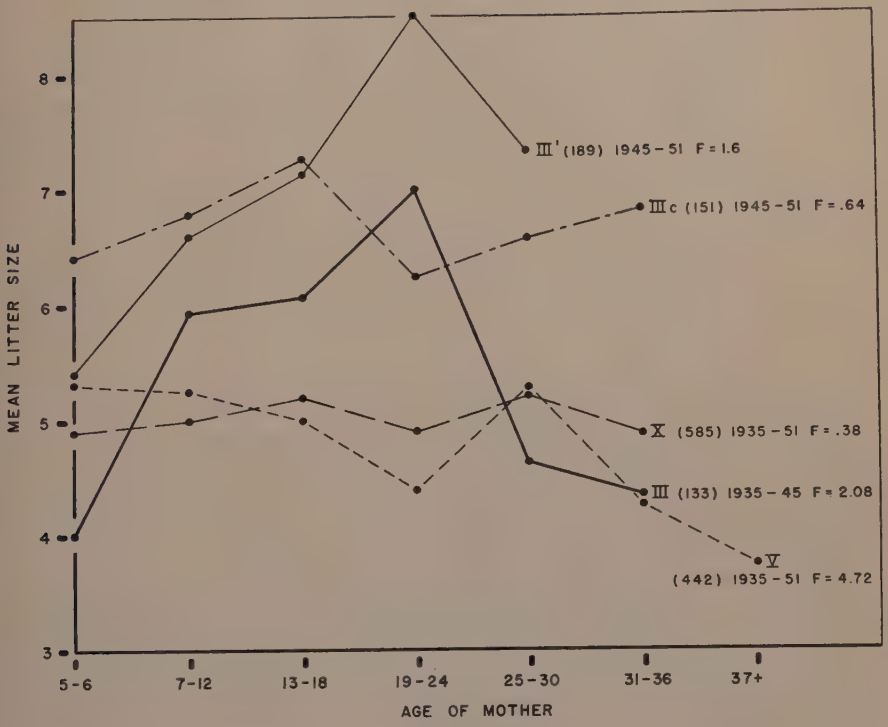

FIGURE 5.

autopsy and by laparotomy. It has been further observed that, in 15 out of 16 cases of mothers with two or more unsuccessful matings, infertility has been due to inability to ovulate. P'an (1940) reported that complete thyroidectomy reduces gonadotrophin potency of the anterior pituitary, and this has been confirmed by Chu and You (1945) and Chu (1945), who further point out that thyroid deficiency induces follicular hypertrophy, an observation also made by Hoffmeister as early as 1893. Associated with this ovarian irregularity in thyroidectomized rabbits, these authors have also observed prolongation of the gestation period, stillbirths, abortions, and deaths at parturition.

The incidence of stillborn young and deaths at birth have already been stated. Although the difference in the gestation period in these two races is not statistically significant, there is some tendency for the gestation period of race *IIIc*

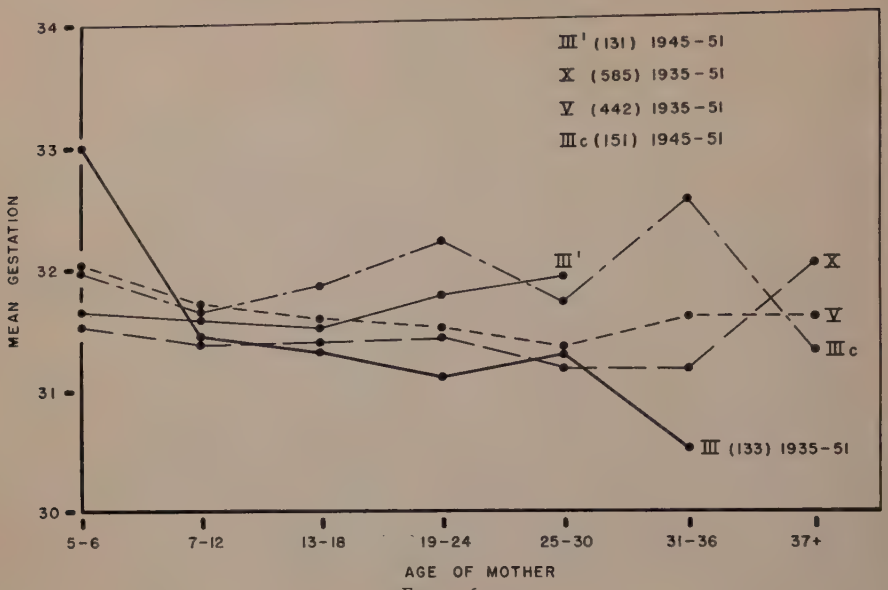

FIGURE 6.

mothers to increase directly with the mother's age; whereas, that of race *X* declines (FIGURE 6). Since race *IIIc* has a greater incidence of prenatal mortality, and a tendency to longer gestation, particularly in later life, these observations could be further evidence of hypothyroidism of this race. When the changes in weight of mothers with age (FIGURE 7) are considered, however, it is found that there is a highly significant decline in body weight in *IIIc* mothers after one year; whereas in race *X* the adult weight achieved at one year tends to be maintained. Since hypothyroidism tends to increase adiposity and thus body weight in humans, a generalized hypothyroidism in race *IIIc* does not seem indicated. It is possible that a temporary thyroid deficiency—as the result of imbalance either of the several fractions of pituitary secretion (FSH, LH, Thyrotropic, *etc.*) or between pituitary, thyroid, and other sex hormones—is influencing the pattern of the reproductive cycle, but it seems unlikely that such an explanation will explain the racial difference in viability of offspring.

In summary, evidence has been presented of inherent differences in viability of offspring between two races of rabbits which are influenced by age of the mother. In race *X*, which is a race of 2400 gms mean body size, the life span is longest when the young are born of young mothers and decreases as the mothers age; whereas in race III*c*, a race of 4000 gms mean body size, there is increase in the life span of offspring as the mothers age up to 18 months, after which it declines.

An effort to correlate the changes in pattern of viability as affected by mothers' age with similar age changes in other characteristics of reproduction, indicates that the racial differences in viability are not associated directly either

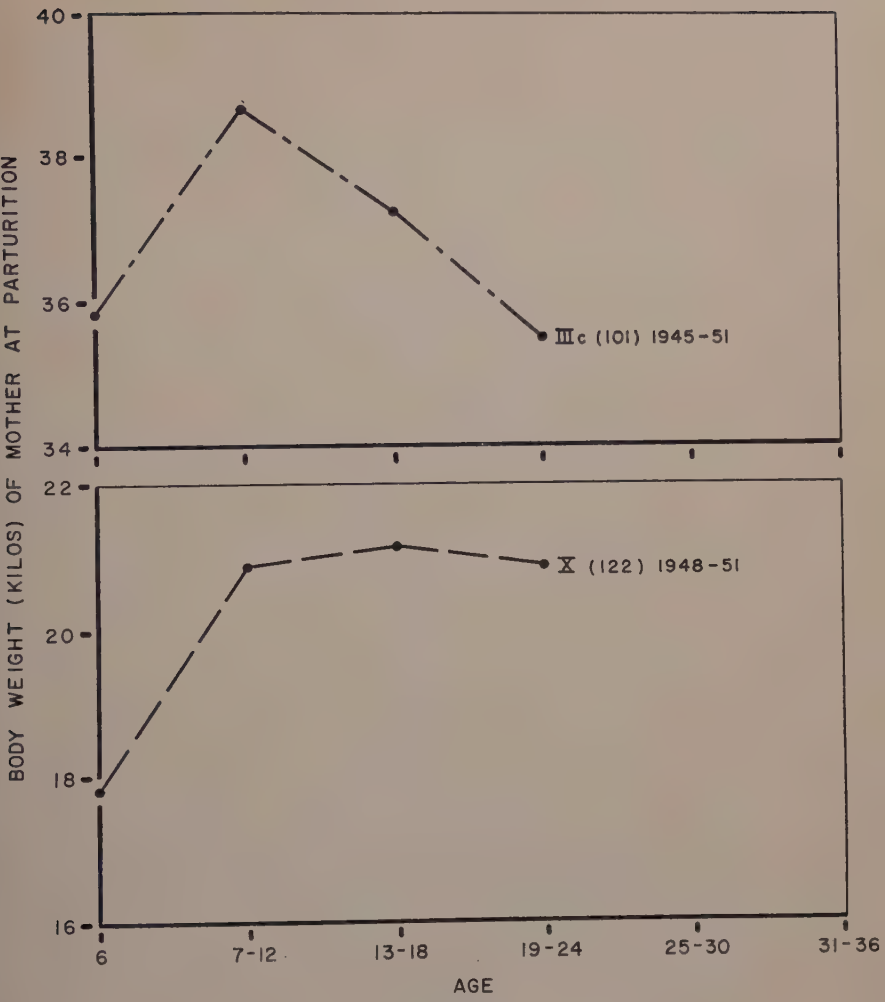

Figure 7.

with age changes in lactation pattern or in weight of mother. Changes in certain maternal behavior characters, gestation period, and fecundity with age of mother, some of which are comparable with changes known to occur in thyroidectomy, suggest that endocrine imbalance might be a factor, but the information at present available is not conclusive.

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MATERNAL INFLUENCE IN EXPERIMENTAL LEUKEMIA OF MICE

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There exists unmistakable evidence that cancer in experimental animals has a genetic basis. The facts related in numerous studies exclude any simple genetic interpretation. Many genes appear to be involved, determining susceptibility to each of the neoplasms investigated. The genes are cumulative in effect and the inheritance of mammary cancer,[1, 2] pulmonary adenomas,[3] and leukemia[4] in mice resembles closely the inheritance of such characters as polydactylism in the guinea pig[5] and various skeletal defects in the mouse.[6] The expression of the character depends on the segregation of multiple genes in conjunction with a threshold of manifestation.

Certain stigmata characteristic of this type of inheritance are quite obvious in genetic studies of cancer. The character is generally sensitive to influences of the environment, either intrauterine or acting later in life. Differences in the expressivity of fibrosarcomas in mice,[7] of mammary cancer,[8] and of leukemia[9] appear directly related to changes in maternal physiology.

One method of detecting the influence on a character of differences in maternal physiology is by the use of reciprocal matings. MacDowell and Richter[10] first observed differences in the incidence of leukemia in reciprocal F_1 hybrid mice. Offspring of the cross ♀ C58 (high-leukemic) × ♂ STOLI (low-leukemic) gave an incidence of leukemia of 61.9 per cent and the reciprocal cross, using low-leukemic mothers (♀ STOLI × ♂ C58) gave a lower incidence, 42.5 per cent. These differences appeared among both males and females, ruling out sex-linkage. Length of life of leukemic and nonleukemic F_1 mice was also found to be strikingly lengthened in crosses using the low-leukemic mother. Since all F_1 mice in these crosses were genetically uniform, the differences observed represent a case of a nongenetic maternal influence on the expressivity of leukemia. This maternal influence was more striking in reciprocal backcrosses where a still further reduction of genetic factors occurs: the incidence of leukemia in crosses of ♀ STOLI × ♂ F_1 was only 19.8 per cent, whereas mice of the cross ♀ F_1 × ♂ STOLI had an incidence of 46.5 per cent. Both leukemic and nonleukemic backcross mice obtained from the cross using low-leukemic mothers lived approximately 100 days longer than mice from the reciprocal cross.

It was reported at this time, but no supporting data were given, that the influence of mothers' milk had been eliminated by reciprocal foster-nursing experiments. No reduction in the incidence of leukemia in C58 mice was obtained nor induction of leukemia in the low-leukemic STOLI strain.

Furth and colleagues[11, 12] observed differences in the incidence of leukemia in reciprocal crosses between their high-leukemic AK strain of mice and two strains in which leukemia is rare, Rf and C3H (See TABLE 1). It is to be noted, however, that in these crosses the differences were confined to the males alone:

TABLE 1

INCIDENCE OF LEUKEMIA IN RECIPROCAL MATINGS OF HIGH- AND LOW-LEUKEMIC STRAINS OF MICE

	Mating combination H/L*					
	C58/STOLI	AK/Rf	AK/C3H	DBA/WA	AKR/NH	C58/C3Hb
	Authors					
	1 MacDowell[10]	2 Cole & Furth[11]	3 Furth *et al.*[12]	4 Law [13]	5 Law[14]	6 Law[14]
	Incidence of leukemia—per cent					
High leukemic strain	89.6	69.3	58	69.7**	85.0	90.0
♀ H × ♂ L	61.9†	21.9‡	50‡	38.5	53.3	45.9
♀ L × ♂ H	42.5	11.6	34	19.7	33.5	44.0

* H = High-leukemic strain; L = low-leukemic strain.
† The maternal influence was even more pronounced in backcross mice, the cross ♀ STOLI × ♂ F_1, giving 19.8 per cent leukemia and the reciprocal cross, ♀ F_1 × ♂ STOLI, 46.5 per cent leukemia.
‡ These differences in reciprocal crosses were found in males only. See text.
** Leukemia induced, in these studies, with methylcholanthrene.

$$\text{♀ AK} \times \text{♂ Rf} = \begin{matrix} 28 \text{ per cent in ♂♂} \\ 15.6 \text{ per cent in ♀♀} \end{matrix}$$

$$\text{♀ Rf} \times \text{♂ AK} = \begin{matrix} 8.8 \text{ per cent in ♂♂} \\ 14.6 \text{ per cent in ♀♀} \end{matrix}$$

and

$$\text{♀ AK} \times \text{♂ C3H} = \begin{matrix} 54 \text{ per cent in ♂♂} \\ 48 \text{ per cent in ♀♀} \end{matrix}$$

$$\text{♀ C3H} \times \text{♂ AK} = \begin{matrix} 28 \text{ per cent in ♂♂} \\ 39 \text{ per cent in ♀♀} \end{matrix}$$

A question of sex-linkage naturally arises in interpreting the results of differences observed in these crosses, since the differences were confined to males alone. This explanation seems unlikely since in another cross to be discussed shortly using a derivative of the AK strain, AKR,* there were obtained differences in the incidence of leukemia in reciprocal crosses in both sexes. It is possible that differences in longevity among males of reciprocal crosses result from a nonspecific influence. This is impossible to determine from the data given by Furth and colleagues since mortality curves for males alone were not shown. This is a problem worthy of further investigation for it raises a question as to the actual existence of a maternal influence in these particular crosses. Reciprocal foster-nursing experiments showed a reduction in the incidence of leukemia in the AK strain, but an induction of leukemia in the low-leukemic strains, Rf and C3H, was not observed. That a specific leukemia-inducing

* This strain was previously designated RIL.[13, 14]

influence, similar to the mammary tumor milk agent, was not responsible for the differences observed in reciprocal crosses was further strengthened by the observations of Kirschbaum and Strong[15] that foster-nursing of several low-leukemic strains, C57BL and CBA, by high-leukemic F strain mothers did not increase the incidence of leukemia.

In 1948 MacDowell and Taylor[9] were able to show that the maternal influence responsible for the difference in incidence of leukemia in reciprocal crosses between the C58 and STOLI strains consisted of a definite and specific resistance contributed by the low-leukemic mother. The resistance-influence was not found in STOLI females at earliest sexual maturity but became increasingly potent with advancing age of the mother. For example: the incidence of leukemia among F_1 female mice of the cross ♀ C58 × ♂ STOLI was 85.3 per cent and of the cross ♀ STOLI × ♂ C58, when low-leukemic mothers were young, average parturition age of 14 weeks, 82.6 per cent. Thus, when resistance is absent, genetic influences show full dominance whether introduced into the cross by the C58 mother or father. Old low-leukemic mothers, average parturition age of 36 weeks, contributed a resistance which decreased the incidence in F_1 females to 56.8 per cent and increased the length of life of leukemics from 567 to 786 days (TABLE 2). It may be seen that the first leukemic F_1 mouse in the group having young mothers appeared some 250 days before any in the group having old mothers. Forty-two per cent leu-

TABLE 2

DISTRIBUTION, INCIDENCE AND AGE AT DEATH OF LEUKEMIC F_1 FEMALES (♀ STOLI × ♂ C58) FROM YOUNG AND OLD STOLI MOTHERS*

Length of life in days	Mothers†	
	Young	Old
300	1	—
350	4	—
400	5	—
450	5	—
500	8	—
550	9	2
600	10	1
650	7	5
700	4	6
750	4	4
800	1	4
850	4	7
900	—	13
950	—	3
1000	—	3
1050	—	1
1100	—	1
Total no. mice	75	88
Per cent leukemic	82.6	56.8
Mean age at death in days	567	786
Difference 25.8 ± 2.7; P = <0.001		

* Adapted from data of MacDowell & Taylor.[9]

† Average age of young mothers at parturition 14 weeks; of old mothers, 36 weeks. Same fathers used for both crosses.

TABLE 3

INFLUENCE OF AGE OF RESISTANT MOTHER AND NURSES (STOLI) ON INCIDENCE OF LEUKEMIA AND AGE AT DEATH IN F_1 (STOLI x C58) FEMALES*

	Mother—Nurse											
	1 Young—Young†			2 Young—Old			3 Old—Old			4 Old—Young		
	No.	Per cent	Age at death	No.	Per cent	Age at death	No.	Per cent	Age at death	No.	Per cent	Age at death
Leukemics	156	80.1	578.6	72	66.6	817.5	95	55.9	805.6	58	69.9	749.1
Nonleukemics	39	—	609.7	36	—	800.6	75	—	810	25	—	721.7

* Data adapted from MacDowell *et al.*[16]
† Average age of young mothers and young nurses 15 weeks; old mothers and nurses average age was 36 weeks

kemics were found in the young mother group before the appearance of any in the older mother group; and at the time the last leukemic died in the young mother group, only 33 per cent leukemics had appeared in the group having old STOLI (low-leukemic) mothers. Longer life was associated with less leukemia, but this relationship is not causal since the difference in length of life is found among nonleukemics as well as leukemics. This influence on longevity then would appear to be nonspecific and not influential in modifying the incidence of leukemia.

That transmission of the maternal resistance factor (MRF) was through the mother's milk was shown by reciprocal nursing experiments. The nurse, either C58 or STOLI, was found to be capable of entirely reversing the proportion of leukemics while only partially reversing the influence on longevity. Further, foster-nurses from another inbred strain, BALB, were found to be neutral, not influencing the incidence of leukemia in reciprocal crosses.

The maternal resistance factor has also been shown to be contributed before birth (see TABLE 3). In the group of F_1 females born of old mothers (average parturition age of 36 weeks) but nursed by young STOLI nurses (average age, 15 weeks), a lower incidence of leukemia is obtained. Compare with the F_1 females having young mothers and nursed by young STOLI strain. It may be seen that the effectiveness of MRF is nearly as great when transmitted by nursing alone (young mothers with old nurses) as when transmitted both before birth and by nursing (old mothers—old nurses).

An explanation for the hybrid difference contributed before birth may be the result of (1) a difference in the cytoplasms of the ova produced by young and old mothers or (2) a difference in the biologic environments furnished the developing embryos by the mothers. There is no way of discriminating these alternatives with the available data, but transplantation of ovaries, in an appropriate genetic setup, as done by Russell[17] may help decide these possibilities.

A similar resistance-influence contributed by another low-leukemic strain of mice, the NH strain[18] has been observed in crosses with the high-leukemic AKR (RIL) strain. The incidence of leukemia in AKR mice, in our laboratory, is nearly 90 per cent with the mean age at death from leukemia of 7.5 months for males and females alike.[14] The majority (97 per cent) of leukemias

TABLE 4

LEUKEMIA IN RECIPROCAL F_1 HYBRID MICE OBTAINED BY CROSSING HIGH- (AKR) AND LOW-LEUKEMIC (NH) STRAINS

Cross	Age of mothers	Sex	No. offspring	No. leukemic	Forms of leukemia*					% Leuk.	Age at death	
					Ly.	Hodg.	PC	RCS	Gran.		Leukemic	Nonleuk.
1 ♀ AKR × ♂ NH	<32 wks.	♀♀	37	23	13	8	1	—	1	62.2	16.8	14.9
		♂♂	40	17	16	—	1	—	—	42.5	16.2	16.0
			77	40	29	8	2	—	1	53.3	16.5	15.5
2 ♀ NH × ♂ AKR	<32 wks.	♀♀	58	24	15	8	—	1	—	40.0	19.8	19.6
		♂♂	40	12	12	—	—	—	—	30.0	16.3	17.0
			98	36	27	8	—	1	—	36.8	18.4	18.5
2a ♀ NH × ♂ AKR	>32 wks.	♀♀	22	7	2	3	—	2	—	31.8	23.1	21.7
		♂♂	20	4	3	1	—	—	—	20.0	20.5	17.4
			42	11	5	4	—	2	—	26.2	21.9	19.7

* Ly. = lymphocytic leukemia; Hodg. = Hodgkin's-like lesion; PC = plasma cell leukemia; RCS = reticulum cell sarcoma; Gran. = granulocytic leukemia.

† Average parturition age of mothers in first group 15 weeks, in 2nd group 19.4 weeks, and in 3rd group (2a) 36 weeks.

in this strain are of the lymphocytic form.* We have not observed leukemia in the NH strain. TABLE 4 shows the incidence and forms of leukemia observed in F_1 mice obtained by reciprocal matings between these two strains and the age at death of leukemics and nonleukemics.

A statistically significant difference in incidence of leukemia is found: 16.5 $\pm$ 7.3 per cent where $P < 0.01$ in both sexes of F_1 mice in the reciprocal crosses. Contrary to the findings with STOLI strain, a lower incidence is found at earliest sexual maturity in crosses using the low-leukemic NH mothers. The average parturition age of high-leukemic mothers in cross 1, TABLE 4, was 15.0 weeks; and of low-leukemic mothers, cross 2, was 19.4 weeks. At earliest sexual maturity there was also observed an increased longevity of both leukemics and nonleukemics in the cross with young, low-leukemic mothers. With increasing parturition age of the low-leukemic mothers, there is obtained a decreased incidence of leukemia and a further increase in longevity of these mice. The average parturition age of cross 2a, TABLE 4, was 36 weeks. F_1 offspring obtained from NH, low-leukemic mothers 40 weeks of age, or older, at parturition showed a still further reduction in incidence to 16 per cent. As in the C58 × STOLI cross, reduction in the incidence of leukemia was direct, and not secondary to the effect on longevity; for longer lives were associated with less rather than more leukemia. FIGURE 1 gives a comparison of the distribution of leukemic deaths as a cumulative percentage in F_1 hybrids having STOLI and NH mothers, contributing MRF.

* Doctor Thelma B. Dunn, Laboratory of Pathology, has kindly made final diagnoses of all the neoplasms in this and subsequent crosses discussed in this manuscript.

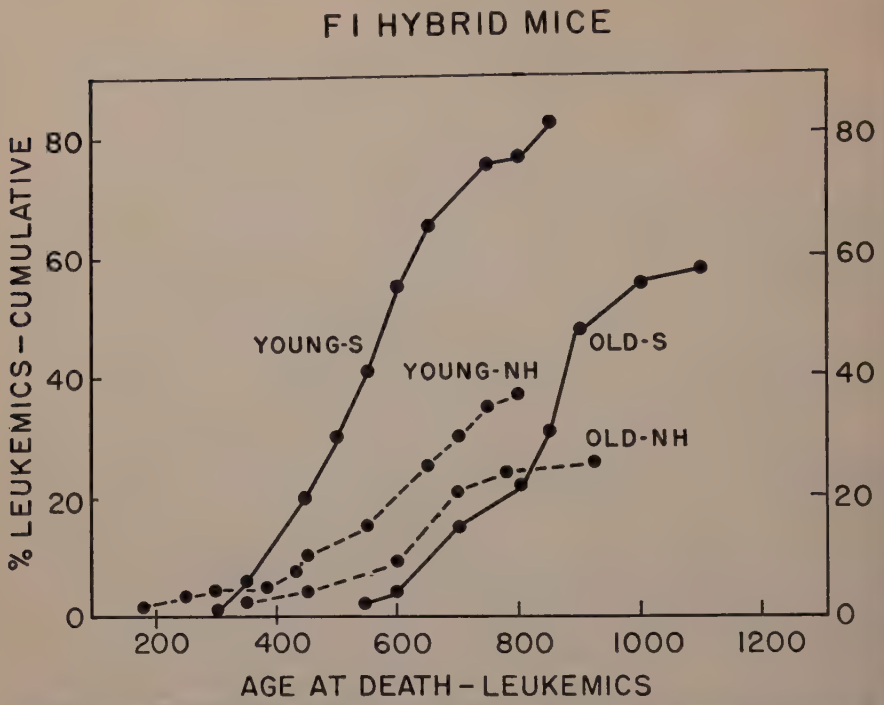

FIGURE 1. Age distribution of deaths from leukemia in F_1 hybrid mice shown as cumulative percentage of total. S = STOLI mothers; NH = NH strain. Average parturition age of young–S mothers 14 weeks; of old–S mothers 36 weeks; of young–NH mothers 19.4 weeks; of old–NH mothers 36 weeks. Age distribution of F_1 leukemics in reciprocal cross ♀ C58 × ♂ STOLI may be superimposed upon young–S curve, whereas a significantly higher incidence of leukemia and earlier mean age at death is found in the reciprocal cross ♀ AKR × ♂ NH. Data on S curves adapted from MacDowell and Taylor.[9]

Foster-nursing of NH mice by AKR mothers did not induce leukemia in these mice so that in all probability NH mothers contribute an MRF similar to that of the STOLI strain. Experiments are now in progress to determine the relative contributions before birth and through nursing alone.

It is of interest to note the occurrence of other morphologic forms of leukemia in the F_1 offspring of this cross, especially a Hodgkin's-like lesion described by Doctor Thelma B. Dunn of this Institute as a pleomorphic lesion consisting of large, round, and fusiform cells which are occasionally multinucleate and large and small lymphocytic, plasma cells, and occasional neutrophilic and eosinophilic granulocytes. There is evidence at present which makes it appear that these lesions are neoplastic. Of 20 lesions of this type found among F_1 offspring, 19 appeared in female mice, at an average age of 19.3 months.

In still another cross between the DBA/2 strain, which develops acute lymphocytic leukemia following painting with the carcinogenic hydrocarbon methylcholanthrene, and the low-leukemic WA strain, a striking difference in incidence of leukemia was observed in reciprocal crosses[19] (see column 4, TABLE 1). The F_1 mice constituted the first and second litters only of both high-

TABLE 5

LEUKEMIA IN RECIPROCAL F_1 HYBRID MICE OBTAINED BY CROSSING HIGH- (C58) AND LOW-LEUKEMIC ($C3H_b$) STRAINS

Cross	Sex	No. offspring	No. leukemic	Forms of leukemia				% Leuk.	Age at death	
				Ly.	Hodg.	RCS	Other*		Leukemic	Nonleukemic
♀ C58 × ♂ $C3H_b$	♀♀	64	27	23	2	1	1	42.2	14.2	19.6
	♂♂	58	29	18	7	3	1	50	20.7	23.7
		122	56					45.9		
♀ $C3H_b$ × ♂ C58	♀♀	43	15	9	4	2	—	34.9	18.7	20.3
	♂♂	82	40	29	5	6	—	48.8	22.4	24.1
		125	55					44.0		

* Including a granulocytic and plasma cell leukemia.

and low-leukemic females. Thus, it is apparent in these results that something present at earliest sexual maturity influences the appearance of spontaneous leukemia. Like the experiments reported by Furth and colleagues, the design of this experiment was not suitable to characterize the influence fully as has been done for MRF in the STOLI and NH strains.

The C3H strain of mice, or $C3H_b$ subline[20] used in this study, is practically free of spontaneous leukemia.[12, 21] In crosses of the $C3H_b$ subline with the high-leukemic C58 strain, in which STOLI maternal resistance factor is effective, no differences in F_1 hybrids as to incidence of leukemia could be detected (see column 6, TABLE 1, and TABLE 5). F_1 hybrid mice born of young $C3H_b$ mothers, average parturition age 14.5 weeks, had an incidence of leukemia of 45.2 per cent while F_1 mice born of old $C3H_b$ mothers, average age at parturition 31 weeks, had an incidence of 42.3 per cent. The distribution and incidence was strikingly similar to that shown in F_1 hybrids of the cross ♀ C58 × ♂ $C3H_b$. The low-leukemic $C3H_b$ strain thus behaves like the BALB strain, described by MacDowell and Taylor, in being neutral. The absence of any MRF in our subline of $C3H_b$, in contrast to that shown by Furth for his subline of C3H, may be due to subline differences, as now recognized for the C3H strain. It is of interest to note the higher incidence of leukemia among males in this particular cross while in the cross involving the AKR strain a higher incidence was obtained in females. In neither C58 nor AKR mice, in our laboratory, is there any striking sex-difference in the incidence or age at death from leukemia.

Specific characteristics of the maternal resistance factor (MRF) are not known. In one of the crosses described, where a difference in the incidence of leukemia in reciprocal crosses was observed (DBA/2 and WA strains), it is known that such differences were not detectable in the reciprocal F_2 generations. In this respect the maternal effect resembles the transitory influences described by Grüneberg[6] for skeletal defects in the mouse. This would appear to be fundamentally different from "maternal transmission" whereby the mother

hands on a self-reproducing entity to her young, such as a normal cytoplasmic constituent or a viruslike agent like the mammary tumor milk agent.

MRF has been shown to be effective in hybrid mice. Since, at least in the case of STOLI mice, it is known to be milk-transmissible, it will be of interest to determine if it is operative in reducing the incidence of pure strain high-leukemic mice. MacDowell *et al.*[16] have indicated that it is effective in decreasing spontaneous leukemia in C58 mice. However, data have not been published in full to date.

It is also of importance to determine whether MRF operates directly on leukemic cells, inhibiting their growth and division, or whether it modifies the conditions under which transformations occur from normal to neoplastic. This problem may be approached either by the use of transplantable leukemias, assuming that MRF is effective in this situation, or by the bioassay technique used by Furth and Boon.[22]

Age of mother is correlated with other variables, such as age of father and birth rank, or parity. It is clear from the experiments of MacDowell (see TABLE 2) that age of father is relatively unimportant in the reduction of leukemia in test mice. The influence of birth rank, parity, has not been determined in either the NH or STOLI strains since only multiparous females have been observed so far as contributing MRF.

Summary

A maternal resistance factor (MRF) has been shown to be effective in reducing the incidence of lymphocytic leukemia in mice. This factor, which is found in certain low-leukemic strains, consists of a definite and specific resistance to leukemia. In certain strains of mice it is shown to be influential at earliest sexual maturity; in others it becomes evident later. A progressive decrease in the incidence of leukemia occurs with advancing parturition age of the low-leukemic mother. It is contributed both before birth and through mothers' milk, at least in the STOLI strain of mice. Some of the characteristics of MRF are discussed and future approaches suggested.

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THE INFLUENCE OF MATERNAL AGE ON IMMUNITY OF OFFSPRING: SOME GENERAL CONSIDERATIONS

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It is quite clear that in certain populations statistically defensible relations can be obtained which indicate a significant variation of the mortality rates for offspring with the age of the mother.[34, 40, 41] A jump from this to the conclusion that the age of the mother is the sole or even the significant causative variable, in any physiological sense, is not immediately warranted, however, without further analysis.

The most interesting and general biological problem is, of course, the relationship between maternal age and the survival characteristics of the offspring, and while a brief outline of this general problem will be given below, our attention as an immunologist will be directed to the more specific problem—the ability to resist infectious disease.

Resistance to infectious disease is itself a complex of associated characteristics, involving anatomical, genetical, hormonal, and humoral components such as antibodies and complement.[11, 14, 29, 39] Even a modest discussion of these interrelationships would lead us too far afield and greatly exceed the time permitted. In brief, it may be summarized by saying that while it cannot be denied that there is such a thing as general good health or an ability to withstand a variety of stresses of at least moderate intensity, there are also important elements of specificity, perhaps best expressed by the familiar folk adage, "The bigger they come, the harder they fall." In more scientific language, we mean an individual can well stand certain stresses, including many infectious diseases, but be quite, perhaps unusually, susceptible to others. It is with this specificity that the immunologist deals. He can account for much of the resistance picture in terms of antibody, complement, and phagocytes; there are other elements which elude his analysis. He can also, by selective breeding, increase or decrease the resistance of a given strain of animal to certain specific infectious agents,[14] but he is hard put to it to explain why. An interesting special case has been described by Hill, Hatswell, and Topley,[15] who found that mice bred for resistance to the soluble endotoxin of *S. typhimurium* are actually more susceptible to infection with the living organism than are normal mice.

Our immediate problem is then the relationship, if such exists, between the age of the mother and immunity in the offspring. Before examining some of the evidence, it may be well to delimit the problem and to define some of the terms. Resistance to infectious disease may be either actual, as exhibited by altered morbidity or mortality rates in the face of environmental risks, or potential, as measured by some serological index, such as antibody levels. The latter, although sometimes an important or indeed indispensable component in immunity are often not the sole factor and may, indeed, be at times misleading as indexes of immunity.

As to the time element involved, the most general statement of the problem would obviously involve a consideration of the immunity status of the offspring over their entire life span. While the progeny will undergo new experiences which will change their immune status, we should be interested in any possible maternal influences on their abilities to respond to such influences. For practical purposes, however, we will limit our immediate interest to a much shorter period, which for humans would be the first year, and for animals an even shorter time. As to the ages for the human mother (assuming her word to the investigator as correct—a rather doubtful assumption at times), we can take the years from 15 to 45 as containing 99.7 per cent of the births, with 90 per cent of the latter occurring between maternal ages of 20 to 40 years.[41]

In approaching the general problem of the relationship of maternal age to infant immunity we can begin by setting forth three biological factors which might influence this relationship:

1. The ability of the mother to produce anatomically complete and physiologically normal offspring. Too great variations in the final product may preclude survival in any environment, and lesser variations decrease the chance for survival under some specific stresses, such as infectious disease.

2. The ability of the mother to manufacture and to transmit to the offspring the specific antibodies necessary for resistance during early infancy.

3. The ability of the mother to transmit to the offspring infectious organisms, antigens, or other agents which may induce in these offspring either a pathological condition or a specific immunity.

We may now inquire into each of these factors in more detail, examining the evidence for their working and, where possible, the extent of their influence. For human material, some idea of the result can be obtained from a study of the appropriate statistics, although due caution must be taken to distinguish situations in which age is associated with various changes and, therefore, gives rise to statistically valid correlations, from situations in which age exerts, through some physiological mechanism, a causative relationship to the changing survival status.

As an example, we may examine the rates for infant deaths for the period 1915 to 1940 (TABLE 1).

This table gives the pattern for the entire United States and illustrates the relative importance of the first month and the first day in the pattern of infant mortality. These are, of course, over-all figures, detailing death from all causes. A more detailed breakdown by causes is given in TABLE 2. It will be noted that prenatal and natal causes account for 60 per cent of the deaths, with disease most of the remainder. During the first month anatomical and physiological defects account for an even larger percentage, as is detailed in TABLE 3, which illustrates the relative unimportance of infectious disease during this period.

The possible associations of maternal age with pathologic states in the offspring is, of course, a well-recognized problem, as evidenced not only by the subjects of other papers in this monograph but also by a number of other published studies. Recent illustrations include the investigations of Thompson[33]

TABLE 1
U.S. INFANT MORTALITY RATES (DEATHS PER 1,000 LIVE BIRTHS)[35]

Year	First year	First month	First day
1915	99.9	44.4	15.0
1920	85.8	41.5	14.8
1925	71.7	37.8	15.0
1930	64.6	35.7	15.0
1935	55.7	32.4	15.0
1940	47.0	28.8	13.9

TABLE 2
CAUSES OF INFANT DEATHS FIRST YEAR, U.S., 1942[35]

Total, prenatal and natal		62%
Premature birth	31%	
Malformations	12	
Birth injury	10	
Others	9	
Respiratory diseases		15
G. I. diseases		8
Epidemic & communicable diseases		2
Other, including unknown		13

TABLE 3
CAUSES OF INFANT DEATHS, FIRST MONTH, U.S., 1942[35]

Total, prenatal and natal		86%
Premature birth	46%	
Malformations	13	
Birth injury	16	
Others	11	
Respiratory diseases		5
G.I. diseases		2
Other, including unknown		7

on celiac disease; of MacMahon[17] on congenital malformations of the heart; of Carter[8] on other malformations; and finally that of Norton[23] on the incidence of neurosis as influenced by maternal age and birth order. These studies are cited not only for their possible value to those interested in special problems, but for their utility to all concerned with the general problem since they illustrate in some detail the approaches necessary for the collection of data and the statistical treatments which must be made to separate some of the variables, such as birth order, birth interval, stillbirths, *etc.*

Although little further reference will be made below to the high incidence of premature births and neonatal or infant malformations, the point must always be kept in mind in approaching group statistics which may include an appreciable percentage of such cases, with marked variation among the different age groups.

Let us now return to our main theme. Having examined very briefly maternal influences on the infant anatomical development, we can consider the possibility of variations in the physiological development of infants which bear

some causal relations to maternal age. Precise separation of such factors from anatomical abnormalities is neither possible nor necessary.

Such considerations will affect our main thesis in the following way. Resistance to disease in the newborn and the infant appears to stem from two distinct mechanisms. The first is passive immunity afforded by antibody derived from the mother. Since reference to this mechanism will be made below, it will suffice to say here that in the absence of antibody the infant, even the newborn, may be susceptible. Pneumonia, syphilis, chickenpox, malaria, and a variety of diseases may be taken as examples.[20, 31, 36]

There are, on the other hand, whole groups of organisms to which the susceptibility of the host is almost completely conditioned by age. Although there has been for some time now a considerable literature devoted to it, the subject has recently been again brought into prominence by activities in the Coxsackie virus field,[21] since this virus, like many other neurotropic viruses, "takes" only in very young animals.

Since the recent work in this active field has been ably summarized by Sigel,[32] reference will be made here only to the more important features which are pertinent to our theses. Thus the studies of Sabin and of others have pointed out definite anatomical and physiological changes, largely specific for each virus, which are associated with the change from susceptibility to resistance during growth. In contrast to this, there are numerous viruses to which infant animals are less susceptible than are slightly older animals. In many cases this change in susceptibility comes about during the weaning period. Where the milk contains protective antibody, it is, of course, tempting to ascribe the loss of resistance to a diminution in the supply of such antibodies. While this is undoubtedly true in a number of instances, further analysis of other cases, particularly a pox disease among rabbits, indicated that this may not be the sole cause. Also pertinent, as Sigel has pointed out, was the striking lack of cases of poliomyelitis among young Eskimo children during a recent epidemic. The latter offered some unusual features in that, although the nursing period among these peoples is unusually long (up to 3 years), most of the adults are highly susceptible and therefore cannot be fruitful sources of antibody.

Even this passing reference to the problem of maternal influences on the ability of the infant to withstand environmental influences should be sufficient to indicate that there are still many factors which have not yet been adequately examined, and experiments yet to be done.

Of special import here would be an analysis of the extent to which maternal influences can condition the general constitution or ability to withstand stress during the later lifetime of the offspring. These factors are, of course, very difficult to separate from the later environmental factors, such as diet, presence of microorganisms, *etc.*, which may profoundly influence susceptibility and resistance. Apart from the genetic components referred to above, very little, indeed, can be said of maternal influences on resistance beyond the newborn stage.

We will next turn to the second of our points listed above, the ability of the mother to influence resistance in the offspring by the manufacture and trans-

mission of protective antibodies. Here the immunologist is on surer ground, for he now deals with material which he can examine in the laboratory. It must again be added, however, that no matter how precise the estimate of the concentration of antibody so transmitted, it is often a major extrapolation to assume that this particular antibody will be concerned with resistance, or even if it is, that the degree of resistance will be even roughly proportional to the antibody alone and not be influenced by other factors.

One of the first reports on the passive transfer of immunity from parent to offspring was that of Paul Ehrlich (1892), who showed that the offspring of female mice which had been immunized against the toxin ricin were themselves relatively resistant to this material. The principle involved was confirmed within the next few years by Theobald Smith, working with antidiphtheria immunity in the guinea pig, and by Hadley, with immunity to Pasteurella infections in the rabbit.

These workers established the following points (summarized by Hill[14]):

(1) The mother animal is able to produce resistant young for as long as two and a half years following the course of immunizing infections;

(2) The duration of immunity among any particular litter is much more shortlived, persisting for not more than 6 weeks after birth;

(3) No immunity resulted in the offspring if only the male parent was immunized;

(4) Immunity was not transmitted to the F_2 generation, *i.e.*, the grandchildren of the immunized female.

In the course of his investigations, Smith also commented, incidentally for his purpose but of major interest for ours, that all the litters of a given mother appeared to have the same range of resistance.

Although it is clear from the work of experimental epidemiologists such as Webster, and Topley and Wilson that there are important genetic components in resistance, or to some extent separately from this point, in the ability to respond to immunization by antibody production, no genetic components are necessarily involved in the passive immunity experiments just mentioned. These can be accounted for solely on the assumption that immunization (or natural infection) produces in the mother's serum specific antibodies which are retained in the body for an appreciable period, and that some of this antibody is transferred to the offspring through the placental circulation, or via colostrum or the later milk.

The transmission of antibodies and the relative importance to the various species of the differing methods of transmission has been extensively studied.[9, 18, 27, 29] A detailed analysis of the literature to 1941 is given by Perla and Marmorston,[29] and the recent literature on antibodies in milk has been reviewed by Marrack.[18]

Representative findings are given in TABLE 4 which illustrates the correlations which have been made between the numbers of layers of tissue between the maternal and fetal circulations, and the passage of antibodies, although other investigators have stated this to be an oversimplification of the problem. In those species having a large number of layers, colostrum and milk become the important vehicles for the transmission of antibodies. Among rodents, the

TABLE 4

RELATIVE IMPORTANCE OF PLACENTAL VERSUS MAMMARY ROUTES IN PASSIVE TRANSFER OF ANTIBODY[19]

Species	Tissue layers	Placental	Colostrum
Pig	5	—	+++
Ruminants	4	—	+++
Carnivores	2	±	+
Rodents, apes, man	1		±

mouse appears to be an exception in that the milk transmission of antibodies assumes a greater role than would appear to be indicated from the table.[1]

In the human, although antibodies can be detected in both colostrum and milk, their concentration is not appreciable unless the serum levels are quite high.[18] The important route of transmission is therefore via the placenta. As will be noted shortly, however, this does not imply that all antibodies are transmitted equally well.

Although these observations, and others to be presented shortly, are important to the present theme in outlining a mechanism by which a given level of antibody can be transferred from mother to offspring, no specific observations are at hand which indicate any variation of this transferring ability with age. There undoubtedly exist data which, if properly tabulated and correlated with age data, could be used to illuminate this question.

There is, on the other hand, abundant evidence that the antibody levels in the mother may vary with time, and our next task will be to inquire not only into the characteristics of this variation but to suggest how this may give rise to some correlations of age and passive immunity.

There are three ways in which antibodies arise in the maternal serum.[5, 25] The first is as a result of natural infection. Unless other factors intervene, the content of such antibodies will depend to some extent on the time which has elapsed since the infection. The second way is as a result of artificial immunization with a particular antigen. The level here is particularly susceptible to a decline with time. Finally, we must include a third category of so-called natural antibodies, of which the isoagglutinins are important instances. These are normally produced by mechanisms which are not yet clear. Their concentrations may however, be increased, or in the case of the Rh antibodies be initially induced, by artificial immunization.

With regard to the immunizability of populations of different ages, there appears to be general agreement among investigators that adults respond best, with a lesser, although for most clinical purposes, adequate response even from infants as young as two or three months.[3, 10, 24, 25] Finally, there is at the extreme of age, perhaps, some lessening of this response.

The measurement of immunizability as a separate property is actually a more difficult matter than is generally realized. Some of the data brought forward in this connection, particularly the levels of antibodies at various ages, reflect many variables, including previous contacts with the agents, opportunities for recent contacts, *etc.*, and the observed age variations may reflect

these rather than a true change in the immunizability status. Any declarations on inherent immunizability must, of course, be statistical in character since there is a considerable range in individual responses to any given antigen. In addition, the poor responder to one antigen may do well to another antigen, and *vice versa*.[13]

In summary of this section, we do not feel that the present quantitative data on immunizability permit any suggestion of true age-dependent variations over the age range in which child-bearing occurs.

Although we may then regard the inherent *ability* to respond to a given antigen as essentially constant over the age range concerned, there is no doubt that most surveys of existing antibody *levels* show important age variations, and that in the future this effect will become even more striking. As we believe the following considerations will indicate, this is more properly a variation in *time* rather than a true, physiological *age* variation. The consequences for epidemiology and public health, if not for theoretical human biology, are nevertheless much the same as if these were true age variations.

Surveys of antibody levels at various ages of the population reveal a number of types, for which reviews and interpretations have been given.[5, 25] It appears that the titer of the so-called natural agglutinins to a variety of microorganisms such as the pneumococci, *E. coli*, *S. aureus*, *etc.*, normally present in the environment, is rather low in children but increases with age. Some curves, particularly those for the isoagglutinins, show a maximum at early adulthood. Others have a marked plateau, decreasing only toward extreme old age. The literature data for skin-test negativity to diphtheria or scarlet fever toxins indicate, typically, a distinct new type, with a slow but gradual rise right up to age 70. There is, finally, a third general type in which antibody is normally absent until a definite time at which artificial immunization is begun, or some unusual infectious agent appears in the environment. Antibodies to tetanus, or among isolated populations such as Eskimos, to certain types of poliomyelitis virus illustrate the last type (TABLE 5). In the cases cited here the viruses were apparently absent from the village for the past 20–40 years, and antibodies to them were not present for transmission to offspring.

We have stated previously that one of the important ways in which antibody is acquired is as a result of infection, either overtly or by subclinical contact. The widespread prevalence of antibodies to poliomyelitis virus among our American population is a case in point. We may inquire now what happens to this antibody. Does it persist?

The evidence from immunization with *killed* vaccines or toxoids is that, in the absence of additional booster stimuli, it does not. In view of the well-recognized anamnestic response, the individual retains, however, the ability to respond quickly to booster doses even at such times as the circulating antibody has dropped to negligible values.[5, 25] With antibodies to tetanus the initial retention may be only two to three years at most. The longer persistence of antibodies to diphtheria has been accounted for by the assumption of subclinical contacts with the organism, or some antigenically similar, if avirulent, variant.

The persistence of antibodies to many virus diseases, particularly the typical

Table 5

Time of First Appearance of Neutralizing Antibodies in Population of Eskimo Village[28]

Poliomyelitis virus strain	Age (years)
Lansing	20
Brunhilde	30
Leon	40

childhood diseases chickenpox, measles, mumps, *etc.*, offers some special problems. Although there are, in most environments, opportunities for renewed booster contacts with the agents cited, this is not always true. The case can be materially strengthened by consideration of antibody persistence to viruses such as yellow fever, which have sharply defined distributions. Immunity in such cases persists life-long, even though the individual may have been removed for years from regions where the virus exists.

Immunologists are still not agreed whether this denotes persistence of the antigenic agent within the body in antigenically active but not infectious forms, or whether antibody can continue to be made in the absence of antigen, contrary to the usual experience with initially nonliving antigens.[5, 25]

Not all antibodies exhibit the same pattern, however, as is illustrated by the persistence of neutralizing antibodies in contrast to the rise and decline of complement fixing antibodies to type 2 poliomyelitis virus.[22]

The foregoing survey has given us some basis for appreciating that the concentration of antibody in a given individual can:

(1) decrease, for certain agents, in some proportion to the elapsed time following infection or other contact with the agent, or

(2) increase again after renewed contact with the agent, or

(3) remain stationary for long periods of time even in the absence of the agent from the external environment (but not necessarily from the host).

We can now turn to examine the relation of this to our main problem as to whether there is an influence of maternal age on the immunity transmitted to the offspring.

The direct relationship would be established if we had any real evidence at hand which indicated that age, through some physiological mechanism, influenced the antibody response in some significant manner. As we have noted, the facts do not warrant such a conclusion at this time, at least over the age range under discussion.

The evidence for a more indirect relationship is more cogent. The principal point to be examined is whether the antibody level is changing with time, so that an apparent age-influenced distribution is being set up.

The clearest, although not the only evidence for this comes from an analysis of the changing pattern of immunity to diphtheria, one of our best studied diseases. The extensive study of Zingher[42] on the variation of Schick-positive reactions with age indicated that, after the initial rise in the first year consequent on the loss of maternally transmitted antibody, there was a continuous drop in susceptibility through age 70. It was believed that this increase in

TABLE 6

VARIATION OF AGE DISTRIBUTIONS OF SCHICK-POSITIVE INDIVIDUALS (SUSCEPTIBLES) WITH TIME

Subject ages	Percentage of Schick-positives	
	Zingher (1923)[43]	Pappenheimer *et al.* (1949)[26]
20	18	50
20–24		39
25–29	12	37
30–34		37
35	11	36
50	8	
70	6	

immunity arose through frequent contact with the organisms in the environment. Some of his data (1923) are given in TABLE 6 with, for comparison, data for the central age range as given by Pappenheimer, Edsall, and their co-workers,[26] in 1949. Other recent data,[37] confirms this downward trend, although data for more highly selected groups can be found which is either higher or lower than that quoted. The example chosen is presented, not only because it is representative of the distinct upward trend in susceptibility, but because the subjects, in military establishments, were a representative cross-section of the general population in these age groups. Although it is fully realized that susceptibility to attacks of diphtheria is not wholly dependent on the Schick status alone, the titrations of susceptibility have a comparative value here which is wholly suitable for the purpose.

Although there is evidently a marked increase in susceptibility among adults, the reverse trend can be noted among infants and young children in many sections of the country, in consequence of the routine immunizations practiced upon these groups. There has been, along with this, and many believe in large part in consequence of it, a marked drop in the rates for diphtheria as a childhood disease. In view of the latter, many observers believe that the opportunity for keeping up the immune status by frequent subclinical contacts will largely be lost. Such a situation has become a recognized danger in any extensive immunization program, although a detailed discussion would be beside the point here. We need only press the suggestion that with the young adults reverting more and more to a condition of susceptibility, the antibody levels which can then be transmitted passively to their offspring will also obviously tend to decrease. This in turn makes it necessary to focus attention on protection of the newborn, a group which in earlier years enjoyed some brief status of immunity to this disease.

It is not without some interest that immunization programs may be creating, to a slight extent, a pattern quite reversed from the above with respect to immunity to tetanus. As is well known, antibodies to the causative organism are not normally found in humans. With the increasing use of tetanus immunizing courses in pediatric practice, however, coupled with booster doses after injury at later ages, some basis for a widespread immunity is being established.

This process has been intensified for the present theme by the mass use of tetanus toxoid in the armed services although, except through the young women who served in such forces, this contribution of immune antibodies to the newborn is probably quite insignificant, since immunization of other adults is not yet routine.

No outline of this character would be complete without some reference to pertussis, which is of special importance here because of the great susceptibliity of the young infant and the relatively high mortality rate. The present state of our knowledge concerning the immunology of pertussis is, however, woefully inadequate and confusing. There appears to be a real immunity to the disease since second attacks are rarely reported. Antibodies to the organism have been reported to be present in some 50 per cent of all pregnant women;[16] nevertheless there is still some doubt whether such antibodies are a true index of protective value.

In view of the importance of the disease in young infants, there have been a number of advocates[16] of the immunization of women with pertussis vaccine during pregnancy, with the objective of increasing the concentration of antibody for passive transfer. This course has been opposed by others on the grounds that too high a concentration of passively transferred antibody will interfere with the development of subsequent active immunization, by vaccination, at a time when the infant must be prepared to meet even greater environmental risks.[30] This problem is, of course, not confined to immunization against pertussis alone (having been debated for diphtheria and tetanus as well[10, 24]). It would appear that some suitable compromise could be reached by proper adjustment of the immunizing dosages.

Although some of the problems presented by the material just given on the changing patterns for diphtheria, tetanus, and pertussis immunizations could profitably be discussed at greater length, we believe enough has been suggested to enable the reader to picture the situation, and to extrapolate to other diseases in which he may be interested. It may be summarized by saying that the patterns of certain diseases are changing, that part of this is due to immunizations on a large scale, another part is due to mass chemotherapy, which may affect not only the immunity resulting from an attack of the disease, but also the general carrier rate and thus the opportunities for subsequent booster doses. To all of this must be added other important influences: some, such as the effect of improved hygiene and sanitation on the decline of typhoid fever are readily appreciated; others, which have affected the course of diseases such as syphilis or tuberculosis during the past few centuries, can only be guessed at.

To complete our cycle of inquiry on the effect of changes in the maternal level of antibodies upon the level of passive immunity in the offspring, we need information on two other points. The first is the quantitative relationship between antibody levels in the two organisms, mother and child. All of the studies on this problem have indicated a close correspondence; as, for example, in the Schick reactivity of the two. More detailed information is given by a recent study of Barr, Glenny, and their co-workers[2] which demonstrated that the concentration of diphtheria antitoxin, at birth, was about 39 per cent

lower in the infant's blood than in the cord blood. Since the latter was elevated some 50 per cent above the normal maternal blood antibody level, as determined either shortly before or after delivery, there would appear to be an almost equal distribution in antibody concentration between mother and child. Some antibodies, such as certain typhoid and streptococcal agglutinins, do not appear to cross the placenta.[12] Since this is not a function of age, it will not be discussed further.

The study of Barr's just referred to also provides information needed for the final question of this section, which is concerned with the rate of loss of antibody passively acquired by the infant. The measurements of these authors indicated a logarithmic rate of decline, with a half-time of four and a half weeks, *i.e.*, a loss of 14 per cent of the antibody per week. This agrees well with the half-time of 30 days found by Weiner[38] for Rh antibodies. As might be expected, the duration of an effective level of passive immunity is dependent on the initial antibody level which, in turn, corresponds to the maternal level at the time of birth.[25]

Before concluding this presentation, we should like to refer to two statistical studies on the general subject of our thesis. The first, by Yerushalmy,[41] published in 1938, is a study of the neonatal mortality among the 2,563 infants who died during the first month, out of a total of 82,140 infants born in New York State (exclusive of New York City) during 1936. The tabular arrangement lists the mortality rates for each age range of mothers, and for the various birth orders, so that these effects can be separated. Aside from the significant differences in infant mortalities at the various maternal ages, this detailed study also contains the interesting observation that, from the infant's viewpoint there is also an optimum age for the father—a point that gives us some cause for reflection!

The second paper, by Woodbury,[40] was published as a report to the U. S. Children's Bureau in 1925, and must be something of a classic in its field. Although based on an exhaustive survey of eight cities between the years 1911 and 1916, it is in some respects superior for our purposes to data which could be collected later, since the use of prophylactic immunization, chemotherapy, and other complicating factors were then not so widespread as they are today. This study cites infant mortality rates for each month during the first year, against the corresponding maternal ages (TABLE 7). Its wealth of tables permits detailed analysis by birth order, interval between pregnancies, economic status of family, and several other factors which complicate the direct interpretation of the age-mortality pattern. Unfortunately, although the causes of death are broken down to include individual diseases or small groups, this classification applies only to the data as a whole and does not permit a further analysis for each corresponding maternal age group, a necessity if each disease is to be considered as having a potential pattern of its own.

Since death certificates or reports of new cases of disease do not, in most jurisdictions, contain, even today, any provision for entering the birth date of the mother of the child, studies such as those cited must be preceded by the very laborious process of finding and then collating independently-filed birth and death certificates. To be of real value, statistically, these must include

TABLE 7

MONTHLY DEATH RATES,* BY AGE OF MOTHER AND MONTH OF LIFE; INFANTS IN EIGHT CITIES[40]

Age of mother	Live births	Infant deaths	Infant mortality rates	Infant age, months											
				1	2	3	4	5	6	7	8	9	10	11	12
Total.....	22,967	2,555	111.2	44.8	9.3	8.1	8.0	7.7	7.4	6.3	5.8	5.7	5.3	3.9	4.5
Under 20	1,584	215	135.7	63.1	11.5	9.5	12.4	7.7	7.0	5.7	5.7	6.4	2.9	2.9	8.7
20–24	6,879	753	109.5	43.5	9.9	7.8	8.2	7.2	5.5	7.0	5.2	5.4	6.9	4.4	3.7
25–29	6,618	671	101.4	42.0	7.4	6.5	6.6	6.3	8.4	5.7	6.1	5.3	4.3	3.3	3.9
30–34	4,231	443	104.7	38.5	9.8	7.7	7.0	10.1	6.9	7.4	5.2	6.2	3.9	2.9	3.9
35–39	2,688	340	126.5	50.2	10.6	10.3	10.4	9.3	9.4	4.5	7.0	5.8	5.9	5.5	4.7
40 and over	958	131	136.7	54.3	9.9	12.3	7.9	6.8	10.3	5.8	8.1	7.0	8.3	6.0	8.4
Not reported	9	2	—	—	—	—	—	—	—	—	—	—	—	—	—

* Deaths per 1000 live births.

many thousands of individual entries, otherwise the age categories into which these will be broken down will include too few representatives. No wonder then that we have had so few of such studies. The present reviewer will appreciate knowing of other studies along these lines, published or unpublished.

Other indications of the effect of maternal age on immunity in the offspring are derived from animal experiments. Thus, in addition to the comments of Theobald Smith referred to above, there are suggestions in the work of Bittner[4] that among mice the offspring of the younger mothers are more susceptible to certain tumors. Our colleague, Doctor Duran-Reynals, has kindly called our attention to the recent work of Doctor J. G. Carr at the Poultry Research Center in Edinburgh, Scotland. In a preliminary report,[6] Carr states that there is good evidence that, in infected flocks, chicks raised from eggs laid by young birds are more likely either to contract fowl leukosis or to become carriers than are chicks raised from the eggs produced by older birds. The mechanism here appears to be a greatly reduced transmission of virus through the eggs of the infected older birds. As a practical consequence of this, the Eire government is believed to be preparing legislation to prohibit the use of eggs from younger birds for routine hatching.[7]

Summary

In summary, it is clear from these varied types of data on humans and animals that there may be significant correlations between the age of the mother and the survival potential of the offspring. The conclusions derived from this are of definite interest, both from the viewpoints of public health and of sociology. The interpretations are, however, complex, since many variables are involved. Some of the latter, such as birth order or spacing of pregnancies may represent mere associations with age, dictated in part by economic conditions or social mores;[34] others, such as antibody levels in the mother, reflect a time variation in the environment rather than a true physiological age variation. Finally there appears to be some indication of the latter, the clearest evidence being of variations in the ability of the mother to transmit potentially infectious agents to the offspring.

From another viewpoint, some of the factors cited affect the ability of the offspring to survive almost any environment, others operate to affect resistance to quite specific infectious agents. Suggestive as these all are, the need for further data and analysis is quite apparent.

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SEARCH FOR NEW CASES OF PARENTAL AND SEASONAL INFLUENCES UPON VARIATIONS WITHIN INBRED STRAINS*

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This monograph has offered extremely interesting material from a wide variety of organisms, including mice, of differences in the nature of offspring attributable to age differences in the mothers producing them. The numerous inbred strains of mice (now more than 100) offer particularly rich material for further search for such characters. Although all of the individuals within one inbred strain have identical heredity (barring a tiny residue of residual heterozygosity or the very rare event of new mutation), and are much more reliably like each other than are random-bred mice, there are variations. That they are nongenic is demonstrable by complete lack of correlation between incidence in parents and offspring. It is analysis of factors contributing to nongenetic variation that sometimes, but by no means always, discloses examples of the types of maternal influence in which this group is interested.

Certain established facts about mouse life histories are of importance as a background to these studies. One is that, in the usual mouse colony, a female may have as many as eight litters before she is a year old, by which time her best reproductive period is over. This first litter is usually born before the female is three months old (average age at first litters in C3H/Jax mice, 74 days; in C57BL/6, 88.5 days), and thus before she has quite completed growth and perhaps before all of her immunity reactions have been completely developed. Murray[1] and Bittner[2] demonstrated for the DBA and A strains that first litters are smaller than second, third, or fourth, and that litter size drops off after the fourth litter. While the pattern of sequence of litter sizes is not identical for all strains, from recent data on the inbred strains in the inbred nucleus of the Jackson Laboratory it appears to be universally true that the first litter is smaller than the second (FIGURE 1). This small initial litter size is probably always due to a smaller number of eggs ovulated, by the young nonparous ovary, as established for four inbred strains by MacDowell and coworkers.[3,4] They found a continually increasing number of corpora lutea with successive pregnancies even up to the 10th. In spite of this, litter size universally decreases in late litters. The only possible conclusion is that the mothers become less efficient in maintaining pregnancy with increasing age and parity. There is evidence of difference between strains in uterine efficiency,[5] and at best it cannot be said that the reproductive efficiency is as high as would be desirable to the investigator. As for factors other than maternal age which can affect the characters of offspring, it is worth recording that, in our colonies at least, matings are made in equal numbers at all months during the year, and it is thus possible to sort out effects of season of birth and season of birth of parents. While environmental conditions are kept remarkably constant through-

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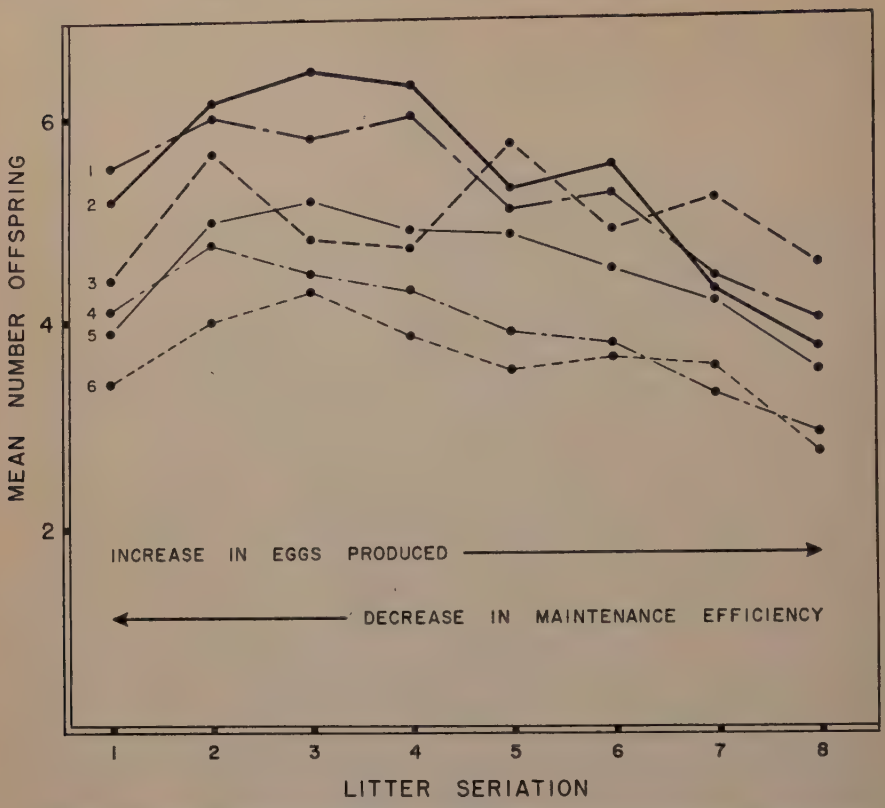

FIGURE 1. Mean size of first to eighth litters in six inbred strains in the Jackson Laboratory inbred nucleus. 1 = C3H/Jax, 2 = C57BL/6, 3 = BALB/c, 4 = DBA/1, 5 = DBA/2, 6 = A/He.

out the year, there are fluctuations in breeding and in incidence of certain diseases worth analyzing.

At present there is a rapidly increasing body of knowledge of the characteristics and life histories of inbred mouse strains. Every year several hundred papers appear in scientific journals reporting research based on inbred mice. These papers can be gold mines, of course, of pertinent information on characteristics of the strains used, but the organizational problem of finding out all that has been published about any strain, the uses to which it has been put, or the observed variations within each, is very large. It seems worth describing at this point a project, called the subject-strain bibliography, undertaken in the library of the Jackson Laboratory. Types of information contained in all incoming articles mentioning inbred mice are coded out on a punched Keysort card (FIGURE 2) specially designed for this purpose. The scheme was developed by the librarian, Miss Joan Staats, and myself, with the cooperation of other staff members in their fields of interest, by examination of the literature up to 1950. Examination of the main subject headings on the card (FIGURE 2) and of a key (not given) to the numerically sorted minor fields gives a realization of

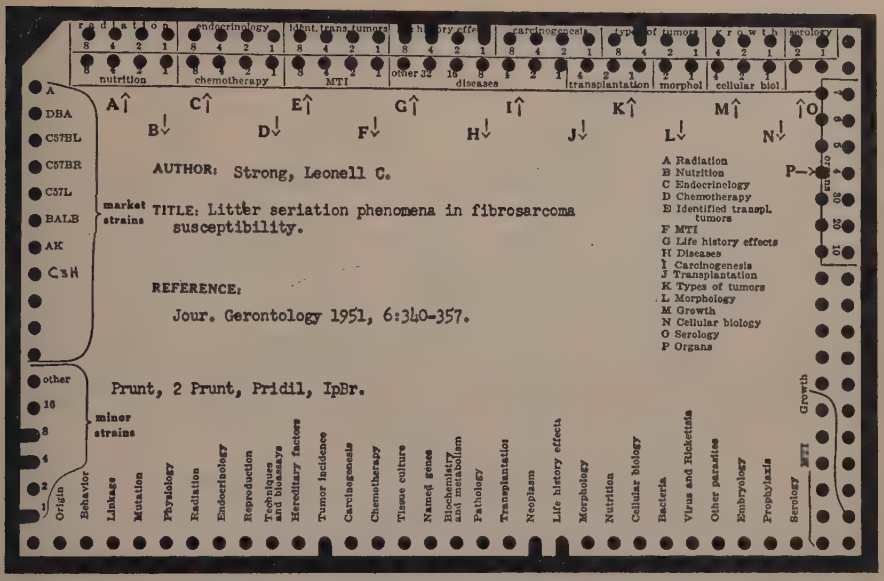

FIGURE 2. Typical reference card in subject-strain bibliography of Jackson Laboratory. This particular reference deals with *life-history effects* on the behavior of a *neoplasm* in the *NH group* of inbred strains. The life history effect is analyzed further in field G as a maternal influence on offspring involving differences in breeding of mothers. The neoplasm involved is identified in field K as sarcoma.

the tremendous scope of current work with inbred strains. The continuing value of the classification has been demonstrated by satisfactory coding of approximately 1000 articles which have come into the library since January, 1950. The field of interest most pertinent to the subject of this monograph is "life-history effects," particularly its subheadings: breeding, general maternal influences, and aging. The proportion of papers with entries under this heading is never high, but they recur regularly. Only six of the 1000 cards since the beginning of 1950 specifically mentioned an effect of maternal age upon characteristics of the offspring. It seems probable that the organization of this monograph will bear fruit in a higher proportion of entries in the papers of 1953-1954.

Almost as cumbersome as tracking down literature references to a rare field is searching through breeding records for possible cases of maternal influence. If one is first interested in a particular type of variation, and seeking to determine its causes, maternal age is one of the possible factors which can be sorted out and tested for. Even to do this, records must have been kept in a special way. But it is quite another matter if you are approaching the question from the other end, looking for characters with significant maternal influence among a wide range of variations. Few investigators have the courage to start looking for the needles in the haystack. Having the responsibility of maintaining in one colony a large number of different inbred strains, of studying their characteristics, and searching for extrachromosomal influences upon their variations, the Jackson Laboratory inbred nucleus group has felt it necessary to seek spe-

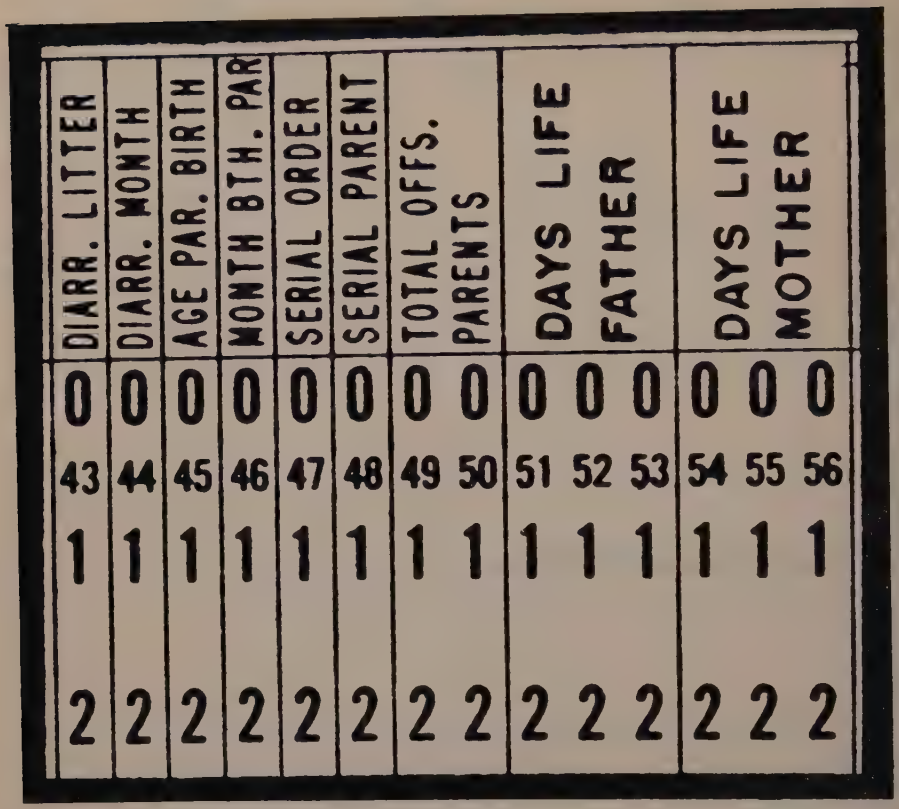

FIGURE 3. Enlargement of a portion of the Inbred Nucleus IBM card, used for recording and analyzing variations occurring within and between inbred strains. Complete life histories from birth to natural death, with autopsy study, are currently available for seven successive generations in most strains. This illustration shows characteristics of the parents of a given individual routinely recorded for future tests of correlation of any variable character with analyzable differences in parents.

cial methods to help them along the way. The use of IBM sorting methods is proving extremely useful in handling our records. The section of the card for each individual called "parental data" (enlarged in FIGURE 3), has removed a large part of the drudgery of searching for maternal age and parity effects, also influences of seasonal or yearly changes in environmental conditions. It has the great advantage that the labor of determining and recording the more easily analyzable maternal variations (age, parity, season), is thus done regularly for all individuals used in the colony, and will always be available to test any suspected correlation of character variation with differences in the mothers. Although our numbers of individuals who have completed their life span has only recently reached proportions large enough for good analysis in most strains, and the sorting of results is just beginning, already a few examples of significant influence have appeared. It seems worth quoting a few of these as indication of types of characters on which evidence of maternal influence might profitably be sought.

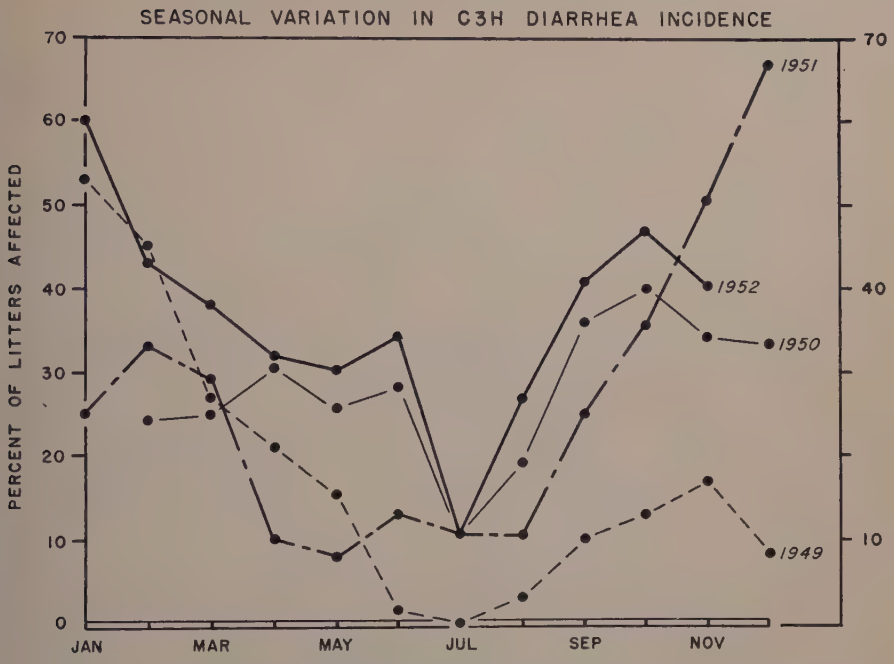

FIGURE 4. Variations in the incidence of juvenile diarrhea in litters born in different months. The proportion of affected C3H/Jax litters is shown for each month from January 1949 to November 1952.

One strain, C3H, is especially susceptible to a juvenile disease, a diarrhea affecting the suckling young.[8] The cause of this disease is unknown to us, but there is a definite seasonal variation in its incidence, fairly well repeatable from year to year (FIGURE 4). The incidence is always low in summer, and extremely high in the late autumn months. Diarrhea data are now available for offspring of 230 females. Whole litters tend to be affected rather than scattered individuals. In addition, offspring of first litters are much more likely to be affected than those in any later litters, the mean incidence, regardless of season, being 31 per cent in first litters, between 10–15 per cent for later litters (FIGURE 5). Appearance of diarrhea in first litters does not materially affect incidence in later litters from the same female, the level being 13.4 per cent of 2nd–4th litters affected where the first has diarrhea, 12.0 per cent where the first litter is free. This unfavorable effect of coming from a first litter has not been explained, and the phenomenon seems worthy of further exploration. It also suggests the possibility of looking for maternal influence on the incidence of other diseases, probably most clearly expressed in juvenile disease.

In another strain, C57BR/cd, the growth of offspring from various litters was followed as part of the control in the testing of a suspected genetic variation. In a population of 321 growing young, there appears to be a correlation between litter seriation and weight at eight weeks postnatal (FIGURE 6). The average weight of females from the first three litters was 17.9 grams, from the 4th–7th litters, 19.2 grams; of males, 20.7 from first three litters, 22.9 from 4th–7th

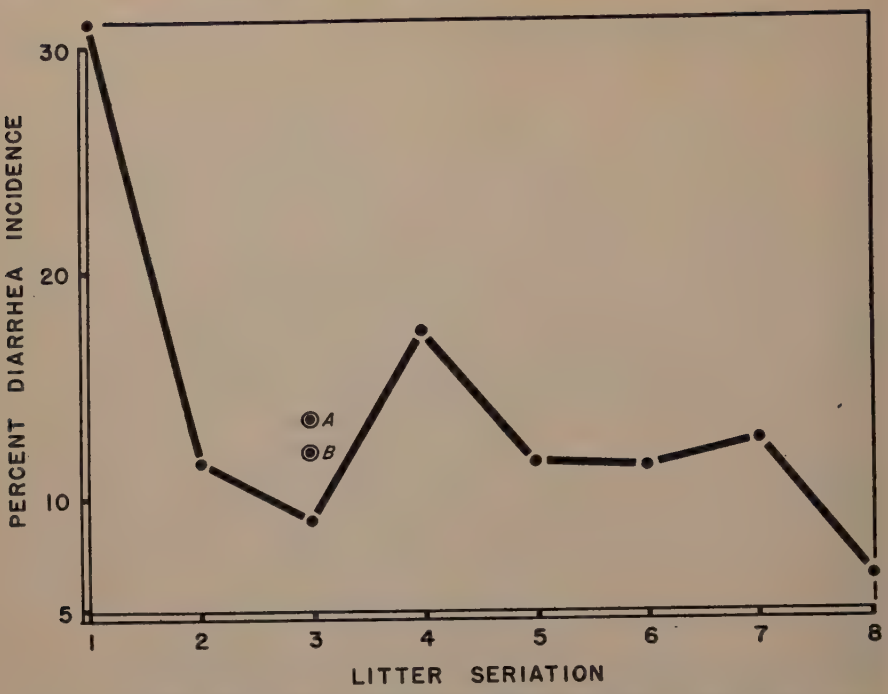

FIGURE 5. Relation of litter seriation to juvenile diarrhea incidence in the C3H/Jax strain. The line graphs shows over-all per cent of 1st to 7th litters affected. A = mean incidence in litters 2–4 of females whose first litter had diarrhea. B = mean incidence in litters 2–4 of females whose first litter was free of diarrhea.

litters. There is considerably evidence[6, 7] that milk supply controls rate of postnatal growth. In this series of animals, there was a steady increase from litter to litter in the weight of the mothers, when their young were two weeks old, from a mean of 26.3 grams with first litters to a mean of 36.4 grams with seventh litters. No attempt has been made to determine to what extent this increase is increment in milk supply or mammary gland development, and to what extent it is increase in body size or general fat deposition. These somewhat limited and incompletely analyzed data suggest that postnatal care of normal offspring may improve with advancing parity of the mother.

The fate of inherently weak individuals may be differently affected by maternal age, as indicated by Murray's study[1] showing increasing incidence of stillbirths and deaths before 30 days in the DBA strain with advancing maternal age. Recent data on the longevity of a specific type of weakling, the *WW* anemic,[9] shows an unfavorable effect of increasing maternal parity. Three different stocks are being inbred independent of each other with selection for high incidence and longevity of this severely afflicted genotype, frequently born dead. (The standard C57BL/6 strain has proven poor for this purpose, giving only 4 per cent *WW*). There is a consistent drop in both aspects of *WW* viability from the first to the fifth litter (FIGURE 7). The parents are heterozygous *Ww*'s, and should produce one fourth *WW* zygotes. The other genes, perhaps

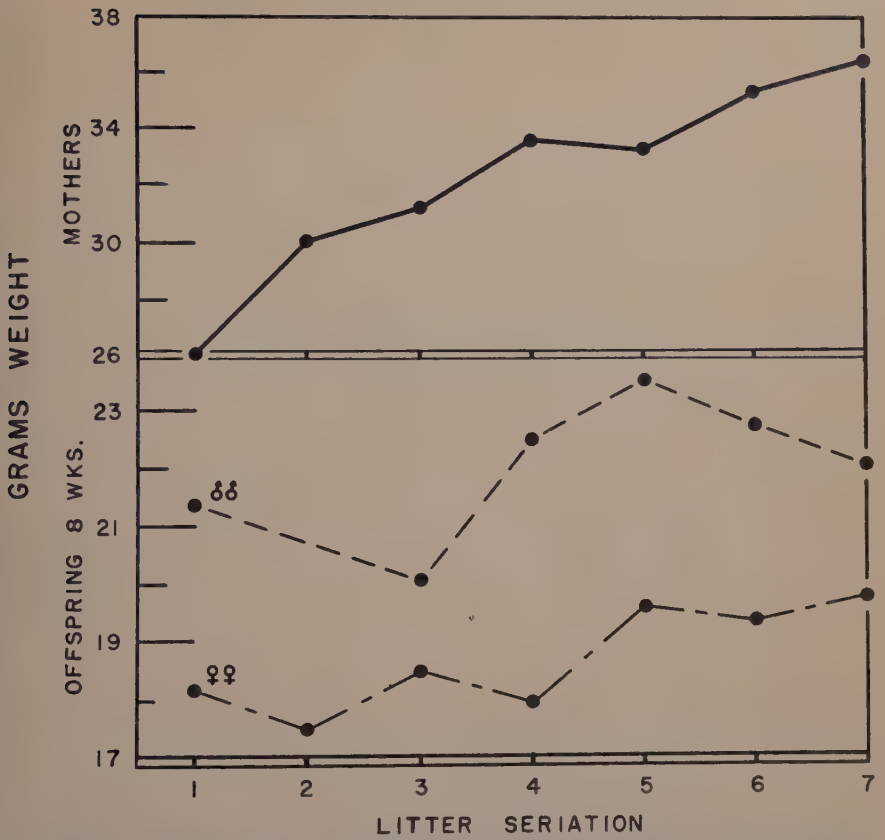

FIGURE 6. Relation of growth rate of C57BR/cd to litter seriation. The lower half of the chart shows the mean weight at eight weeks of females and males from successive litters. The upper half shows the mean weight of their mothers near the end of lactation (two weeks after birth of that litter). Note difference in upper and lower weight scale.

different in each of the three strains, are approaching homozygosity, coming from the 7th to the 12th generation of brother-sister matings (approximately 90–96 per cent homozygosis). In strain I, incidence of *WW*'s observed on the day of birth was 25.2 per cent, the full expected complement, in first litters. The average survival time of these young was 6.88 days. Both incidence and survival dropped rapidly (20. 2 per cent surviving 6.03 days in the 2nd litter, 18.7 per cent surviving 4.92 days in the 3rd, 13.4 per cent surviving 4.04 days in the 4th, and 16.6 per cent surviving 4.04 days in the 5th). Much the same story is found in strain II, with incidence of *WW* born dropping from 20.0 per cent in the first litter to 13.6 per cent in the 5th litter, and longevity of *WW* dropping from 6.66 days in the first litter to 3.91 days in the fifth. In strain IV, where there is considerable other evidence that selection has fixed modifiers making the affliction of offspring less severe, approximately 20 per cent *WW* individuals in all litters, regardless of seriation (1st, 20.6; 2nd, 20.8; 3rd, 21.5; 4th, 17.6; and 5th, 20.6). Even here, however, the *WW* longevity de-

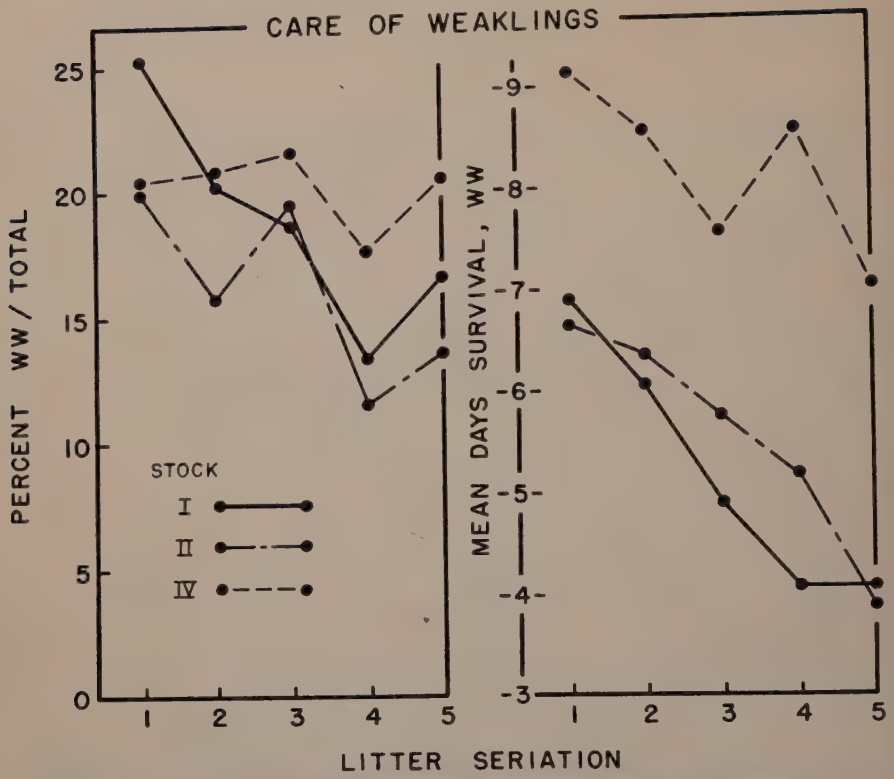

FIGURE 7. The effect of litter seriation upon prenatal and postnatal care of the *WW* lethal anemic. I, II, and IV refer to three different stocks independently inbred 7–12 generations with selection for *WW* survival and longevity. The left hand graph shows changes in prenatal care (drop from genetically expected 25 per cent born) with advancing litter seriation. The right hand graph shows drop in postnatal care, indicated by mean number of days survival of the *WW*'s which are born in successive litters.

pends upon the parity of the mother. The average survival of *WW*'s from first litters in strain IV is 9.13 days, 8.53 from 2nd litters, 7.53 from 3rd, 8.52 from 4th, and 7.0 from 5th. In some way, younger mothers are better prepared to bring *WW* young to term and to keep them alive after birth. It seems quite possible that the decrease in reproductive efficiency mentioned in the introductory section is working particularly harshly upon the weakest embryos. The differences in postnatal survival may be independent of the uterine environment, or may simply be a reflection of differences in prenatal care.

Thus, by picking favorable cases out of many possible types of variation, it has been possible to show examples of effects of maternal parity upon the incidence of a juvenile disease, upon the growth of normal healthy offspring, and upon the pre- and postnatal care of a specific type of weakling. In two of the cases, advancing maternal age has been advantageous, in the third, deleterious. Ways in which maternal age might be expected to alter the characteristics have been discussed briefly, and suggestions made as to how the chosen examples may be related to maternal physiology. Description has also been

given of methods currently in use facilitating finding references to maternal influences in the literature and simplifying the collection of maternal influence data in experimental material.

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THE EFFECT OF ALTERED ENVIRONMENT AND OF AGE ON MOTHER-YOUNG RELATIONSHIPS AMONG ANIMALS

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American Museum of Natural History, New York

For the psychologist working with human subjects, the relation of parental age to offspring behavior has long been a matter of great interest. One need only examine the literature of the clinician to appreciate the complexity of the problem. The relationship of the woman of 40 years of age to her new-born infant and that of the developing child to the mother is frequently marked by traumatic incidents which affect the behavior and the development of the young organism. Even growth and physiological health are impaired when the normal expressions of love and affection are inhibited by repression and distortion accompanying the feelings of age differences.

In the field of comparative and animal psychology, however, the experimental implications of age relationships have been largely neglected. Few investigations have dealt with the topic of this monograph for reasons which will be sketched briefly in subsequent discussion. Before describing what has been accomplished in research on age in the field of animal behavior, it is advisable to introduce some methodological considerations which affect experimental productivity in this area.

Age has been a relatively ignored parameter in psychological investigation except in so far as it is restricted to the so-called formative period of infancy and childhood. Much attention has been paid to the effect of early experience upon later adult behavior. Scott[7] and the other participants in a conference on this topic have presented an inclusive research program on both the human and animal levels which repays reading by all interested in comparative research on age factors. This emphasis has been considerably influenced by Freudian hypothesizing and by the need to seek experimental validation for the concept of childhood's importance. Significant research has pointed to the necessity for adequate longitudinal studies in which the origin of adult behavior patterns can be seen in early infancy. Papers by Schneirla[5] and Riess[3] at a symposium on field methodology in comparative psychology document this point of view. Other studies[6] have shown that there are critical periods and unique types of learning in infancy which throw light upon adult behavior. The development of the organism's actions, like that of its morphology, does not appear to occur in a linear function. Smaller quantitative variations seem to accumulate and to result in relatively sharp, qualitative and, frequently, gross modifications of behavior. This finding has been energetically dealt with in many researches. Hebb,[2] in his fascinating and important book on the development of behavior in organisms, initiated the concept of early and late learning as determining differential adult types of adaptation. Scott[6] and his co-workers at Bar Harbor have done much stimulating research on the appearance and meaning of "critical" periods in the maturation of puppies and other animals.

The importance of the studies on the relationship of early experience to adult life has, however, minimized a correlative aspect of the developmental program. Any good longitudinal study must take into account the reciprocal affect of adult behavior on the growing organism as well as the projection of infantile experience upon adult behavior. It is in this field that research involving the age of the parental environment has been lacking. In almost all psychological studies of parent-young relationships, there has been an implicit assumption which may be phrased, "Once a parent, always a parent." Although critical periods have been postulated for the neonate, once developed into adulthood, there have been relatively few inquiries into nonlinear processes in the adult. It is apparent from the few studies that have been done that there are critical periods in the life cycle of the grown organism during which environmental manipulation will affect the young. Rosvold[4] has shown that electro-shock administered at certain crucial periods in the oestrus cycle of the female affects the viability and behavior of the young rat. In this type of consideration and in experimental programs for investigating the origin of adult behavior, it must be kept in mind that there can be no dichotomous separation of factors. If early experiences are projected on to later adult behavior, then it must also be true that these activities constitute one of the variables affecting early life patterns. Longitudinal experimentation and field study is valuable only in so far as it is able to handle its data from a multifactorial point of view.

Even where the reciprocal effects mentioned above are considered, there has been evidenced another type of experimental bias which must, of necessity, have distorted the interpretation of research data. What is referred to here has frequently been called the technique of isolation. In order to determine the effect of age on behavior, the growing organism has been isolated from species-mate contact, brought up by itself for varying periods of time and then comparisons made within the varying groups of isolated organisms. Behind this technique lies the assumption that, in an environment kept as constant as possible, only internal, innate, or maturational factors will emerge. For instance, maternal behavior in the white rat has been called instinctive because it appears upon suitable provocation in the absence of social learning derived from the presence of older and more experienced cage-mates. Experiments will be described shortly which indicate that complicated behavior patterns which seem to appear fully developed in adult life may, in part, depend on a series of simple and apparently unrelated learning situations in early life. The investigator is all too prone to discount the effect of constant and uniform environment because of its stability. So, too, there has been a neglect of the organism's own presence in the field. The isolated animal is never so deprived that it does not experience its own corporeality together with the sensory and perceptual stimulation resulting from its bodily presence. Birch,[1] in a series of studies now drawing to a conclusion, has devised an ingenious method of testing the "isolation" hypothesis. From infancy to sexual maturation, the female rat is furnished with a large rubber collar. When the animals tries to smell, lick, or manipulate its genitalia, the wide collar interferes with any contact. Thus the front half of the organism is isolated from the rear. When

these females give birth to young, no suckling, cleaning, or licking of the neonates has been observed. It is the hypothesis of the study that experience of the adult with its own genital sensory qualities is one determinant of maternal-young relationships. Therefore, before describing any offspring behavior as the result of the chronological age of the parent, it is necessary to ask, what has happened to the parent in its evolution to that age. Perhaps the effect on the behavior of the offspring will then be related only incidentally to chronological age but more relevantly to adult learning and environmental stimulation. For behavioral work, at least, age represents an accumulation of experiential and physiological changes whose specific effects must be adequately identified.

Finally, there are questions of analogic argumentation. To what extent can data from the more popular laboratory animals be compared and be useful in generalizations about age effects? So far, the surveys of published literature reveal an astoundingly small number of species in which the behavioral problems of aging have been studied. Even here the data are derived predominantly from laboratory study without the enrichment and corrections obtained from natural habitat observation. Dorothy Parker's criticism of an actress as having run the gamut of emotions from "A" to "B" is also true of comparative psychologists for whom the biological kingdom consists of ants, rats, dogs, and chimpanzees. It is almost certainly true that the relative restriction of environment in the laboratory will reduce the behavior repertoire of the adult organism and thus obscure the possible effect of age on the behavior of the offspring.

Since much of the biological data on the effects of parental age deal with genetic characteristics, it will be useful to present psychological investigations in an analogous field. Among the types of behavior which have been described as innate, instincts occupy a large place. The experimental study of these so-called unlearned acts is again becoming a fertile research area. In a series of studies under way at the American Museum of Natural History, the ontogeny of maternal behavior in the white rat has been the focus for the application of some of the methodological considerations just discussed.

Maternal behavior has been described as follows. For a short time prior to parturition, the pregnant rat will pick up and heap pieces of paper or other material into what has anthropomorphically been called a nest. Following the birth of the litter, nesting activity is stepped up in intensity. A new type of behavior also appears. This is known as retrieving and consists of picking up and placing the pups in the "nest." Suckling then takes place. The proofs of the instinctive nature of the observed pattern have been twofold. First, it appears in adult animals which have been segregated at an early age and allowed to mature in isolation until mating time. Second, the pattern appears wherever rats have been observed. The application of the term "instinct" to this behavior blinds the experimenter to some of the experimental and physiological variables which may, in part, cause maternalism. In one series of experiments, pups were isolated at 14 and 21 days after birth, brought up in a deprived environment and at various ages of maturity, mated and placed in a standardized testing situation. This latter consisted of a wooden box around the sides of

which were hung pieces of paper spaced at one inch distances. The observation of nest building, retrieving, and suckling took place in this piece of apparatus. The major experimental group consisted of females brought up in an environment from which all manipulatable material had been removed. Powdered food was supplied in fixed food cups. Because of the shape of the cup and the extremely fine pulverization of the food, it was impossible for the animal to handle any amount of it. Water was furnished in immovable bottles. The cage floor was of wide-gauge mesh so that feces dropped through into a receiving-pan. No bedding material of any sort was provided. Animals were brought up in isolation from species-mates. With this as the basic experimental group, other groups were placed under varying conditions of environmental deprivation and age. Some of these groups lived in environments with pellets of food available or with bedding material in the cages. Additional experiments sought the effect of the presence of other animals on the experimental subjects. Finally, and more relevant to the topic of age, the basic group was subdivided into rats mated at 90, and 160 days of age, plus or minus variations due to the stage of the oestrus cycle. Two groups of 160-day-old animals were studied. The first group was kept in a deprived environment for the whole period, the latter having 90 days deprivation followed by 70 days of normal environment. Further comparisons involved the time of onset of deprivation, namely at 14 and 21 days of age.

In general, for the basic experimental group, the effect of deprivation at 21 days upon maternal behavior at 90 days of age is rather dramatic. In these animals there was no nest building, decreased retrieving and infant mortality of 75 per cent due to the absence of suckling. This contrasts with normally processed animals and is most striking. Nest material was used by the experimental animals but not in a nest-constructive fashion. The available paper strips were torn from their holders, carried about and left in haphazard fashion on the floor of the testing chamber. So too, the pups were carried energetically and frequently but very rarely were they gathered into one area.

Comparison of the first experimental group, deprived at 21 days of age, with the animals segregated at 14 days of age shows a much greater degree of impairment in maternalism for the 21-day group. The present status of our research does not yield any explanation for this difference.

Another comparison involved the effect of the earlier and later onset of deprivation on the 90- and 160-day group of mothers. In general, the longer the period of deprivation, the greater the impairment. The older animals, however, the 160-day group which had only 70 days of actual deprivation, were more seriously disturbed by the earlier segregation than by the later segregation. Here is an absolute effect of age which is not explicable at present by any of the data gathered thus far.

Because the number of viable offspring was small in all groups, statistically significant data on the maternal behavior of the pups of the deprived mothers was not obtainable. Qualitative and quantitative impairment of maternal functioning was, however, obvious in the few animals studied. This may be an artifact resulting from impoverished nutrition during infancy.

Although the purpose of these studies is not directly relevant to the topic

of this monograph, the results do bear on some of the considerations advanced in the first part of this paper. Since the maternal efficiency of the adult reflects upon the young animal, it is important to study the factors which may be causative of this behavior. The point to be emphasized here is that the usual conditions of animal care are in themselves variables which affect the behavior of the older animals and, therefore, of their offspring. It is necessary to be on the alert during experiments to detect the influences of such constants as nesting and manipulatable materials before conclusions can be drawn as to the effect of age alone on behavior.

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SUMMARY AND GENERAL CONCLUSIONS

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Scattered evidence for parental-age effects on filial characteristics has accumulated in sufficient volume to justify an attempt at viewing the diverse genetic, embryologic, oncologic, geriatric, biometric, immunologic, and psychiatric data through the collective medium of a working conference such as that on which this monograph is based. Although aging provides a superficial common denominator for the observed phenomena, coordination of the multi-faceted material would presuppose a better factual grasp of the underlying mechanisms than it has been possible to obtain through past experimentation. Not only are there many variable characters completely unaffected by parental age, but age-responsive traits themselves are not always clearly heritable and may be sensitive either to intra-uterine environmental fluctuations or to forces effective after birth. In those cases where genotypically controlled traits fluctuate upward or downward in penetrance with maternal parturition-age (Grüneberg and Searle, Law, Hauschka and Brown, Russell, Strong, Wright), reasonable guesses at the nature of the environmental (?) influence have been made. Among the proposed factors are hormonal changes, cytoplasmic entities, biochemistry of the internal environment, viral titers, antibody levels, and differences in frequency of chromosomal crossing-over with advancing age of the parent (relegating at least some of the responsibility to the aging father who, throughout this monograph has been the forgotten sex).

All the mammalian gentoypes for which significant shifts in penetrance are correlated with parity have one feature in common; they are multifactorial complexes under the influence of plus and minus modifiers, and subject to thresholds of manifestation. A borderline case, for which genetic control is not conclusively demonstrated, is mongolism in man. The incidence of this anomaly is very strongly affected by maternal age, nearly two fifths of the cases being born to mothers over 40 years old. According to Penrose and also Ingalls, a simple Mendelian explanation is not warranted. Among the suggested hypotheses are the following: a specific genetic constitution of wide distribution becomes rarely manifest, usually at a late maternal age; crossing-over or mutation-frequency facilitated in older mothers; a complex antigenic reaction between mother and fetus; a maternal recessive gene; a chromosomal translocation; a cytoplasmic agent; or finally, an environmental accident, such as hemorrhage or a maternal endocrine disturbance.

From Doctor Murphy's account of congenital malformations in man, choice of a young mother would seem to be one's best insurance for being born intact. Similarly rotifers descending from lineages of adolescent mothers have longevity advantages over the clonal issue of older ones (Lansing). Mice, on the other hand, appear to benefit from increasing maternal age, as exemplified both by the downward trend of skeletal defects (Grüneberg and Searle), and by the maternal resistance factor (MRF) in mouse leukemia (Law). One and the

same character, however, might show either an upward or downward tendency or no parental-age response at all, depending on the genetic environment in which it is studied. This was observed by Strong for fibrosarcoma latent periods in different mouse stocks.

Despite shortcomings in clarifying the physiology of parental-age impact on progeny, the contributions to this monograph nevertheless have definite practical implications:

(1) Strong's extensive work on fibrosarcoma susceptibility in mice makes desirable a program of cancer survey designed to demonstrate whether similar age-forces participate in the origin of cancer in man. Increasing cancer incidence might not, as is generally supposed, be largely consequent to greater life-expectancy, but might perhaps be inherent in the equally pronounced decline in family size, *i.e.*, there are fewer offspring from older mothers.

(2) The maternal resistance factor which virtually eliminates leukemia from the litters of older females in certain mouse crosses (MacDowell, Law) suggests a systematic search for possible association between leukemia incidence and maternal age in human vital statistics.

(3) If family histories include records of congenital abnormalities (*e.g.*, mongolism, anencephaly, hydrocephaly, spina bifida, hydatiform mole, chorion epithelioma, central placenta previa, achondroplasia), child-bearing beyond the late thirties had best be avoided.

(4) Residual variability in "inbred" strains may need further re-examination in view of the marked phenotypic differences with regard to such traits as cancer susceptibility, eyelids open, and skeletal variations between sublines derived from early and late litters of one and the same genotypes (Strong and others).

As for future experimental analysis that might conceivably promote our understanding of age factors interacting with genotype, a search for more easily analyzed cases should continue. The most elegant experimental system presently available is Lansing's rotifer orthoclones. Although mouse genetics has nothing comparable to offer, setting aside portions of I.B.M. analysis cards for parental-age data (as has been done by Russell) may pay valuable future dividends. Since continued plus-selection for traits varying with parental age tends to reduce the maternal-age influence (Hauschka and Brown), study of such traits would seem to be most rewarding in the middle range of penetrance.

Direct experimental attack on the mechanisms involved should include: cytological analysis of chiasma frequency in different age groups; transplantation of older ovaries to young recipients and *vice versa;* inhibition or enhancement of penetrance through hormone and a variety of treatments at known developmental stages (*e.g.*, Steiniger, Warkany, Ingalls, and others).

Of special importance are continued studies of differences between progeny from sublines based exclusively on older and younger parents. If such repeatedly derived sublines were to exhibit consistent differences between one another as regards, for instance, longevity (Lansing) or fibrosarcoma susceptibility (Strong), the objection that phenotypic divergencies are caused by mutants occurring during the course of prolonged subline propagation becomes void. Lansing's rotifers are, however, homozygous and parthenogenetic, hence,

phenotypic variations and sperm contributions do not exist. The effect of maternal age on longevity of rotifers is not only cumulative but also reversible. So far reversibility has not been demonstrated in cancer susceptibility.

The botanical contributions to the present problem appear to be clear cut. Blakeslee has shown that the mutation rate is increased by the aging of the seeds. He also demonstrated, from work with Doctor Barton, that humidity and temperature in which the seeds have been aged increase the mutation rate in *Datura stramonium*.

Ashby and Wangermann have shown that successive populations of leaves derived from the same growing point (the meristem) differ progressively and that these changes may be ascribed to an aging meristem. They present a working hypothesis to account for the symptoms of aging and rejuvenation in *Lemna minor*. They conclude that "a clone of *Lemna*, as a whole, does not age. Individual fronds age and die but rejuvenation takes place during vegetative reproduction." Bünning has shown that the characteristics of plants, particularly their germinative characteristics, show seasonal fluctuations and that some of these changes are transmissible. He states that "a seed from an early flower in the spring has quite different qualities compared with a seed from a later flower." He further states, "the processes on which the periodicity of life activity, that is, of periodic aging and rejuvenation is based . . . are not of a biochemical but of a biophysical nature. The periodicity is interrelated with alternations in the colloidal status of the protoplasm."

The sex ratio in man appears to change with maternal age as demonstrated by the extensive analysis of the data on the Japanese race by Takahashi. "When the mothers are 20," says Takahashi, "the sex ratio of births is higher and then the ratio decreases gradually until the mothers are about 40–49. The sex ratio of births by aged mothers (over 50) is the highest, but this observation may not be a biological fact but a social one."

In view of the widespread interest in parental age and characteristics of the offspring, as indicated by the contributions of the authors of this monograph, it appears that the topic is a timely one and that the base line has been drawn upon which further conferences and monographs may be organized.

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